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JUNE, 1932

# Phytopathology

An International Journal

Official Organ of
The American Phytopathological Society

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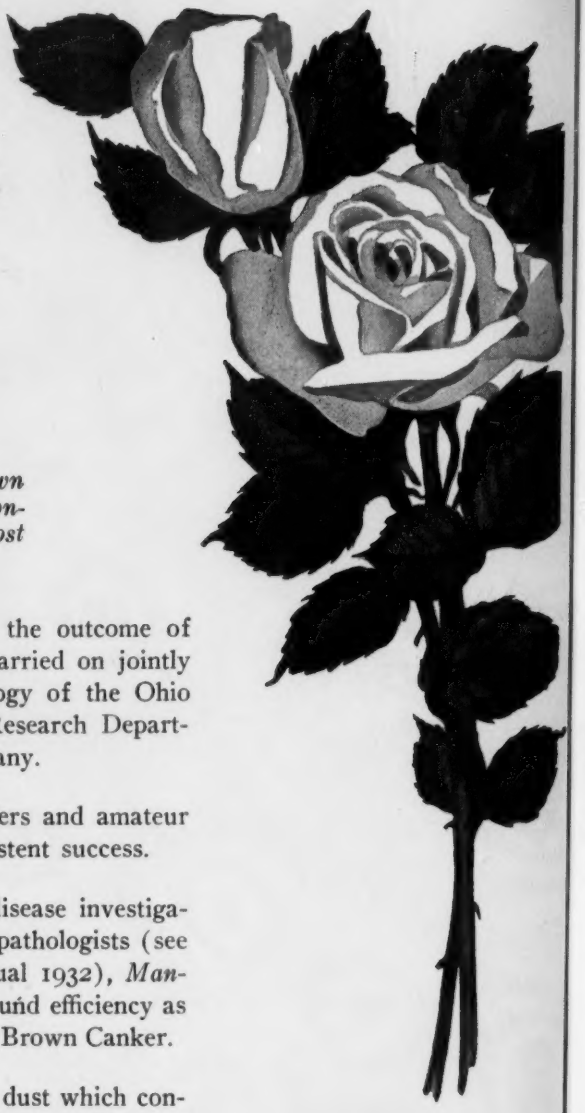

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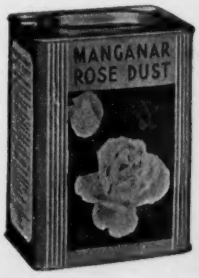


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NATHANIEL ORSON HOWARD

# PHYTOPATHOLOGY

VOLUME 22 JUNE, 1932 NUMBER 6

## NATHANIEL ORSON HOWARD
### 1880–1930

WALTER H. SNELL

Dr. Nathaniel O. Howard, Pathologist of the Division of Forest Pathology, United States Department of Agriculture, in charge of the European larch-canker investigations, died suddenly in Providence, R. I., on September 14, 1930. He was in his 51st year.

Dr. Howard was born in Providence, May 11, 1880. He was educated in the city schools and was graduated from Brown University with the degree of Ph.B. in 1903. He received his Sc.M. degree in 1917 and his Ph.D. in 1925 from Brown University. The Rhode Island College of Pharmacy granted him the degree of Pharmaceutical Chemist in 1929. Dr. Howard was a member of Phi Beta Kappa, Sigma Xi, The American Phytopathological Society, American Chemical Society, and Harmony Lodge No. 9, F. & A. M.; he was Secretary and Treasurer of the R. I. Botanical Club, a Fellow of the American Association for the Advancement of Science, and a charter member of Phi Kappa Psi, his college fraternity.

Dr. Howard married Miss Mae Elizabeth Hayes, of North Attleboro, Massachusetts, in 1903. He is survived by his widow, three sons, Edmond Harrison, Walter Henry, and Nathaniel Orson, Jr., and by three brothers, Charles M. Howard, of Pawtucket, R. I., and Harrison C. and Albert W. Howard, of Providence.

Much of Dr. Howard's work in life was teaching. Following graduation Dr. Howard taught science in Coshocton, Ohio, and Rutland, Vermont. In 1905 he returned to Providence, R. I., to teach chemistry at the Technical High School. This he did until 1918. After 1920 he was for several years instructor in the Department of Botany at Brown University, on a part-time basis, teaching morphology occasionally and handling the work of the department in plant physiology and plant chemistry. He also taught a course in advanced plant pathology as a cooperative project between the Department of Botany and the Government Division of Forest Pathology at Brown University. For several years, he also gave the work in botany at the Rhode Island School of Pharmacy.

Dr. Howard entered upon his pathological work first as a part-time collaborator at the Providence office in 1913, investigating the diseases of

shade and ornamental trees and shrubs. In 1918 he was transferred to the office in Forest Products Laboratory at Madison, Wisconsin, to study the control of sap stain, mold, and incipient decay of wood used for wheel spokes and other parts of war vehicles. This work took him through a good part of the hardwood belt of central United States. Shortly after the conclusion of this work, he studied a disease of camphor in Florida. He returned to the Providence office in 1920 to continue work upon shade and ornamental trees until the spring of 1929, when he was placed in charge of the European larch-canker investigations, in which he was engaged until his untimely death.

A loyal and companionable friend, a capable teacher, a cooperative and faithful associate, a devoted husband and father, a scientist of broad interests and ability, Dr. Howard can ill be spared from among us.

Dr. Howard's more important contributions to scientific literature follow:

The control of sap-stain, mold, and incipient decay in green wood with special reference to vehicle stock. U. S. Dept. Agr. Bul. 1037. 1922.

The relation of an undescribed species of Pestalozzia to a disease of Cinnamomum camphora Ness. & Eberm. (Abst.) Phytopath. **13**: 47–48. 1923.

Notes on chemical injuries to the eastern white pine (Pinus Strobus L.). Phytopath. **12**: 362–368. 1922. (With Walter H. Snell.)

Improved method of sectioning textiles. Textile World. **63**: 195–196. 1923. (With Walter H. Snell.)

The relation of moisture contents of wood to its decay. Science, n. s. **62**: 377–379. 1925. 1923. (With Walter H. Snell and Myron U. Lamb.)

A new disease of Douglas fir. Science n. s. **69**: 651–652. 1929.

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# THE INFLUENCE OF ALTERED HOST METABOLISM UPON MODIFICATION OF THE INFECTION TYPE WITH PUCCINIA GRAMINIS TRITICI p. f. 21

DOROTHY F. FORWARD[1]

The work of many students of rust parasitism since Marshall Ward has led to the acceptance of the type of infection produced by any given physiologic form of rust upon any given host as an index to physiological compatibility or incompatibility between the host and fungus protoplasts, the nature of which remains unknown. This association has already proved useful in an exploration of the extent of physiologic specialization, especially in the cereal rusts. It should be equally useful in studies of the physiological factors contributing to the state of compatibility or incompatibility, provided a technique for its application could be found. Such a technique is described in the present paper.

There has been a tendency, until very recently, to emphasize unduly the constancy of the infection type within a loosely defined range of experimental conditions, and, indeed, to regard the infection type as a virtually unalterable character proper to a genetical strain of the host in combination with a genetical strain of the rust. But from the standpoint of an inquiry into the nature of the physiological processes concerned in the determination of the infection type, it is not constancy within a certain environmental range but modifiability when the range is overstepped that would be useful as an experimental tool. If, by modifying the processes of the host or of the fungus in a manner definable in terms of the physiology of either, one could at the same time modify the infection type, a program for the investigation of the physiological basis of compatibility or incompatibility would be feasible. The present series of experiments was designed as a first step in what must be an extensive and involved program.

Since the physiology of the rust mycelium cannot, with our present knowledge, be studied apart from the host, the best chances of success obviously lie in attacking, first, the contribution of host physiology to the determination of the infection type. The contribution of the fungus was, therefore, standardized as definitely as possible for these experiments by the selection of a single physiologic form, known to be particularly aggressive and stable in its host relationships. On the other hand, the physiology of hosts infected by this rust form was so modified as to cause modification of infection types. In the present paper, a comparison is made of the effects of several types of experimental treatment on infection type. In

[1] Fellow of the Ontario Research Foundation in the Department of Botany, University of Toronto.

future communications, the modifications of host physiology will be defined in respect to those aspects that the results of this work seem to indicate as significant. Then a comparison of the results of experiments similar to the present ones, but involving numerous rust forms, will be of value in elucidating the nature of the contribution of the pathogen toward success or failure in the establishment of compatible relations between the host and fungus protoplasts.

## REVIEW OF LITERATURE

When the experiments reported in this paper were planned, indications already existed that the type of infection produced by one rust form on one host strain is not unalterable, but nothing in the nature of a convincing demonstration of modification of reaction of the host then existed. Since this work was undertaken, however, several such demonstrations have been forthcoming.

In 1905, Ward (29) had reported having cultured *Puccinia glumarum* (Schm.) Eriks. & Henn. on a susceptible variety of wheat in overheated soil and in a carbon-dioxide-free atmosphere. He was able to reproduce on the susceptible host phenomena similar to those observed on "immune" plants. These phenomena he described as "starvation of hyphae in a nest of dead cells, or the corrosion of cells beneath the spores sown on the leaf" —apparently the same phenomena that, in the system of Stakman and Levine (25), distinguish a resistant from a susceptible infection type. While his lack of knowledge of physiologic specialization of *P. glumarum* on wheat may leave some doubt as to the purity of his rust material, nevertheless the fact that control plants in a favorable environment showed only susceptible infection types renders it highly probable that his experimental treatment actually caused modification of the reaction of the host plants.

In 1927, Waters (31), in describing corn plants infected with *Puccinia sorghi* Schw. and subjected to darkness for several days wrote as follows:

> "In the other plants either the infected leaves died, or the region surrounding the sorus appeared to be completely dried out, leaving the rust mycelium isolated from the food in other regions of the leaf."

It is at least possible that the drying out of tissue around the pustule signified the occurrence of hypersensitive tissue and, therefore, a shifting of the infection type as a result of darkening the host.

Not until 1929, after the present investigation was under way, did further evidence concerning the modifiability of infection types come to my knowledge. In 1929, Gassner and Straib (10) published the results of a number of experiments in which the infection type produced by several cereal rusts on their seedling hosts was altered within limits by culture of the hosts at different concentrations of atmospheric carbon dioxide. Rela-

tive differences between susceptible and resistant varieties were not, however, seriously disturbed. Several publications since 1929 show, on the other hand, that with certain rust-host combinations the infection type is subject to quite radical alteration.

Gassner and Straib (11), Waterhouse (30), Gordon (13), Peturson (24), and Johnson (17) have reported instances where extreme changes in the reaction type of one or more strains of wheat or other cereal to standardized rust forms resulted from changes in the temperature at which the host plants were cultured. Melander (20) reported that uredinia of *Puccinia graminis tritici* "developed very slowly at 0–1° C. and sometimes the infection type differed from the normal type produced at 10° and 20° C." Waterhouse (30), Gordon (13), and Johnson (17) also mentioned light intensity as a probable factor affecting the infection type in some instances, but no specific investigation of its importance has been recorded. Hassebrauk (16) reported having modified the infection type produced on wheat seedlings by *P. triticina* by alterations in the mineral nutrients supplied to the seedlings.

Those who have reported changes in infection type appear to have been chiefly interested in their effect on the host relations of the various rust species involved and particularly in their effect upon the identification of physiologic forms. They have not failed to recognize their possible usefulness in a study of the nature of the physiological processes determining the infection type, but almost no experimental work has been done in this direction. Johnson (17) made preliminary efforts to discover differences of structural or chemical constitution between seedlings of the same variety of wheat grown at high and low temperatures but found none. The nature of the relationship of environmental factors to modification of rust infection type, therefore, remains quite unknown.

Environmental factors are known to affect other aspects of rust development, such as the abundance and vigor of infection or the spore form. The consensus of opinion is that where environmental factors affect these aspects of rust development they act chiefly through their effect on host metabolism. This has been demonstrated, at least as regards light, by Mains (19), Gassner (9), and Waters (31). The popular theory of the relation of light to rust development appears to be that the rust is dependent for nutrition upon some substance synthesized by the host in the light. Scant attention has been paid to the possible importance of the catabolic phases of host metabolism in affecting rust development, although Trelease and Trelease (26) have recognized this in relation to the infection of wheat by mildew. Fromme (8) and Mains (19) have described a limited number of experiments in which a period of darkness following inoculation retarded the development of uredinia on susceptible hosts by a period approximately

equal to the duration of darkness. The interpretation placed upon this was that rust development was completely arrested during darkness. The probable cause assigned was the absence of some essential nutritive substance formed by the host only in the light. This conception of the relation of light to rust development appears to have gained popular acceptance. However, very little experimental evidence has been advanced in support of this or, indeed, of any theory of rust nutrition. The true nature of the relation of rust development to host metabolism, like the relation of infection type to host metabolism, remains unknown.

A recent paper by Hanna (15) deals with the relation of host physiology to a general susceptibility to rust. His work constitutes a part of the program of wheat-stem-rust investigation outlined by Newton, Lehmann, and Clarke (21). Hanna's mode of procedure was to measure varietal differences in host vigor in terms of the rates of physiological processes and to search for a correlation of varietal differences in some of the processes with a general susceptibility or resistance to stem rust. He found indications of such a correlation with a high content of carotinoid and chlorophyll pigments but none with diastase or catalase activity or with the rate of respiration over a 2-hour period of leaves of equivalent time age grown in the greenhouse.

## MATERIALS AND METHODS

A single physiologic form of the wheat-stem-rust fungus, *Puccinia graminis tritici* Eriks. & Henn. p. f. 21, has been employed in about 80 experiments in the greenhouse, involving 11 of the 12 standard differential wheat varieties commonly used in the identification of physiologic forms of *P. graminis tritici.* (Kanred was omitted because it seldom shows any signs of infection by form 21). In pursuance of the policy of trying to produce a definable change in host physiology, the experimental treatment chosen was the confinement of infected and noninfected plants in the dark for more or less prolonged periods of time. Some of the metabolic consequences of prolonged darkness have been determined for certain green plants or plant organs (1–7, 12, 14, 18, 22, 23, 27), and it seemed that a technique such as was used to discover these could be profitably applied to a study of the metabolism of the wheat varieties under experimentation here. Three types of experiment have been used: 1. Continuous-darkness experiments in which seedlings in pots were subjected to prolonged periods of darkness after rust infection was established. 2. Experiments with shortened daily exposures to light. 3. Continuous-darkness experiments in which detached leaves were subjected to prolonged periods of darkness.

The experiments reported in this paper were all carried out in the greenhouse and extended over a period of about a year and a half. No

environmental control was attempted, with the exception of the use of artificial light, in addition to daylight, for some of the winter experiments.

The differential hosts employed for determination of physiologic forms of *Puccinia graminis tritici* at the Dominion Rust Research Laboratory, Winnipeg, Canada, were grown from seed of known record, obtained from that laboratory.

Only one physiologic form of rust, p. f. 21, has been employed in my experiments, and only this form was introduced during the course of my investigations into the experimental section of the greenhouse that they occupied. Therefore, practically no opportunity for contamination of the form has been present, but occasional inoculation of the complete series of differential hosts was carried out, in order to verify the purity of the form. Form 21 was chosen as a physiologic form particularly aggressive and stable in its host relationships. Seven out of the 12 differential hosts are very susceptible to this rust form, 1 moderately susceptible, and the other 4 highly resistant (Fig. 1). With form 21 none of the differential hosts gives, under natural conditions, the heterogeneous reaction designated by Stakman and Levine (25) as "x."

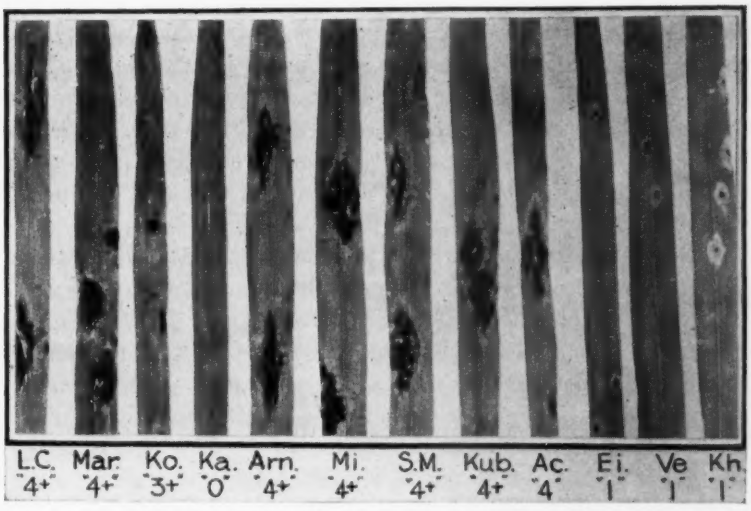


FIG. 1. Reactions of differential hosts to *Puccinia graminis tritici* p.f.21. Photographed 23 days after inoculation. Left to right, 1 leaf each: Little Club "4+", Marquiz "4+", Kota "3+", Kanred (B-2-5) "0," Arnautka "4+", Mindum "4+", Speltz Marz "4+", Kubanka "4+", Acme "4", Einkorn "1", Vernal "1", Khapli "1".

Seedlings were grown in 4-in. pots and were inoculated in the single-leaf stage according to the technique usually employed for the study of stem rust on seedlings. At the time of inoculation a selection was made.

At first, only the evidently atypical seedlings were removed; later, only those were retained that fell within a definite height range. This selection left from 8 to 15 seedlings in a pot. No rigid rule was followed as to the number of plants left in a pot, but observations revealed no differences in experimental results that could be traced to variation in this respect.

In the first three experiments, the development of mycelium in inoculated leaves was followed in free-hand sections, stained with cotton blue in lactophenol. When the mycelium was collecting under the epidermis and the first urediniospores could be seen, the plants were considered ready for subjection to darkness. After the first three trials, it was found that this stage could be recognized from the exterior as the time when small whitish infection flecks on the leaf first become macroscopically visible. Sectioning was accordingly discontinued, and the appearance of infection flecks became the criterion for the stage of development when the plants were ready for transfer to the dark chamber.

At this stage, the endosperm had disappeared from most of the seeds, and many of these had dried up. Certain individuals retained a trace of endosperm, but never more than a trace was observed, although a half-dozen individuals, or usually more, were examined in each experiment.

The dark chamber stood in the greenhouse in which the plants were grown. In the first experiments it was a box 20 in. x 26 in. x 30 in., with ventilation through light baffles, aided by an electric fan in the chamber. Since this chamber would accommodate only about 20 pots, it was replaced by a larger one, 3 ft. x 3 ft. x 4 ft., similarly ventilated. Soon after the large chamber was introduced, use of the fan was discontinued, for it appeared unnecessary.

The temperature of the dark chamber was not controlled in any way. Its daily range of variation was somewhat less than that in the greenhouse. The range varied from about 10° F. in winter to over 30° F. in some weeks of the summer. Temperature changes in both the dark chamber and greenhouse were recorded by thermographs. For the most part, the dark-chamber and greenhouse temperatures fluctuated about approximately the same mean, but that in the dark-chamber failed by 5 to 10° to reach the lower extreme of greenhouse temperature and by 5 to 10° or more to reach the upper extreme. The temperatures of the two environments differed chiefly in range of fluctuations. For this reason and because the temperature differences from one season to another in the greenhouse were as great as or greater than those between greenhouse and dark chamber during any one experiment, it is safe to conclude that the recorded results of confining plants in the dark chamber are attributable to darkness rather than to a temperature change.[2]

[2] This has since been definitely proved with plants grown at constant temperature and subjected to periods of darkness at the same temperature.

Changes from greenhouse to dark chamber and *vice versa* were always made after dark, so that 1 day in the dark means continuous darkness for 2 nights and 1 day, 2 days in the dark means 3 nights and 2 days, and so on.

Except in early experiments, noninoculated control plants and inoculated ones received the same experimental treatment.

Plants, on removal from the dark chamber, were returned to the bench beside untreated controls, both inoculated and noninoculated. Daily notes on their subsequent development were made. These records included information, separately recorded either for each pot or for the two or three pots receiving similar treatment, on the following particulars: The number of leaves bearing open uredinia, the number bearing closed uredinia, those bearing none but with other signs of rust infection, and those showing no signs of infection; the size of the uredinia, their color, and any irregularities of appearance; the estimated proportion of infection centers with uredinia to those without, and the proportion of open to closed uredinia; the presence or absence of chlorotic rings, hypersensitiveness, green islands, or other symptoms of incompatibility; the color and general appearance of the leaves and the amount of dead tissue where any was present. Descriptions of noninoculated plants referred to the color and general appearance of the leaves, and the amount of dead tissue, if any. Plants were kept under observation for at least a month after inoculation, although daily records were not so long continued.

The symbols introduced by Stakman and Levine (25) for the description of types and degrees of infection by physiologic forms of *Puccinia graminis tritici* have been adopted. These symbols were originally intended for use within a certain range of environmental conditions in the identification of physiologic forms of this rust. As convenient shorthand descriptions of the externally visible results of rust infection, however, they may be applied, without violating the definitions set forth by Stakman and Levine, to infection types arising under such a special set of conditions as that arranged for the experiments recorded here. To assist in obtaining a clear conception of my interpretation of these symbols, reference may be made to figure 2.

Since it is important in the present discussion to distinguish between the flecks that always precede pustules and those that represent abortive infections, the former are always referred to as "infection flecks," a term used by Ward (28, p. 276). Abortive infections are distinguished as "necrotic flecks" (Fig. 1, Khapli) or "nonnecrotic flecks" (Fig. 5, B, "4-day" leaves), according to whether a visible area of host tissue is in a collapsed, necrotic state or whether other signs of incompatibility between host and fungus are evident. Nonnecrotic flecks are usually small, whitish areas, definite in outline and quite distinct from the chlorotic areas pro-

duced by rust developing normally on the same host. They resemble an early stage of necrotic flecks, and are definitely hypersensitive although not collapsed and brown. The centers of such flecks may be brown, although no signs of pustule formation are externally visible. Certain small flecks may be entirely brown, but the tissue is not collapsed. These flecks differ distinctly from the ordinary necrotic fleck, but certainly represent abortive infections. The intrinsic relations between the two types of fleck are not understood; the two terms are used simply for descriptive purposes. An attempt is made to be specific as to type whenever flecks are mentioned.

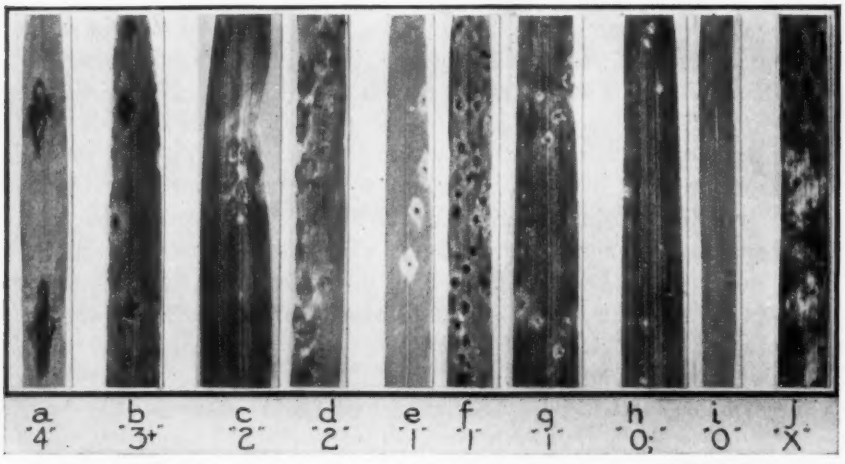


FIG. 2. Rust-infection types. a. "4", Arnautka, Exp. 65, control, photographed 23 days after inoculation. b. "3+", Arnautka, Exp. 64, in dark 1 day, photographed 17 days after inoculation. c. "2", Arnautka, Exp. 31, in dark 2 days, photographed 14 days after inoculation. d. "2", Mindum, Exp. 17b, in dark 2 days, photographed 14 days after inoculation. e. "1", Khapli, Exp. 65, control, photographed 23 days after inoculation. f. "1", Little Club, Exp. 71c, in dark 4 days, photographed 27 days after inoculation. g. "1", Arnautka, Exp. 37, in dark 3 days, photographed 17 days after inoculation. h. "O", Arnautka, Exp. 37, in dark 2 days, photographed 12 days after inoculation. i. "O", (B-2–5) Kanred, Exp. 65, control, photographed 23 days after inoculation. j. "x", Arnautka, Exp. 17a, in dark 5 days, photographed 23 days after inoculation.

## EXPERIMENTAL RESULTS:

### I. EXPERIMENTS ON SEEDLINGS WITH CONTINUOUS PERIODS OF DARKNESS

A. *Conditions of experimentation.* The majority of experiments have been in the nature of subjection of plants to continuous periods of darkness. All the plants of one sowing (except those in the control pots) were placed in the dark chamber at the same time, and lots of 2, or sometimes 3, pots of inoculated plants and the same number of noninoculated ones were removed daily until all had been returned to the light. For example:

| | No. of pots placed in the dark at one time | No. of pots removed after continuous darkness for: | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 day | 2 days | 3 days | 4 days | 5 days | 6 days | 7 days |
| Inoculated | 14 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Noninoculated | 14 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |

The maximum sojourn in the dark varied from 3 to 7 days in the many experiments performed.

About 70 experiments of this type were conducted in the greenhouse between March, 1929, and June, 1930, involving 11 of the differential hosts.

B. *Effect of continuous darkness upon the appearance of host tissue.* Leaves of all the varieties of wheat in the dark chamber, whether infected[3]

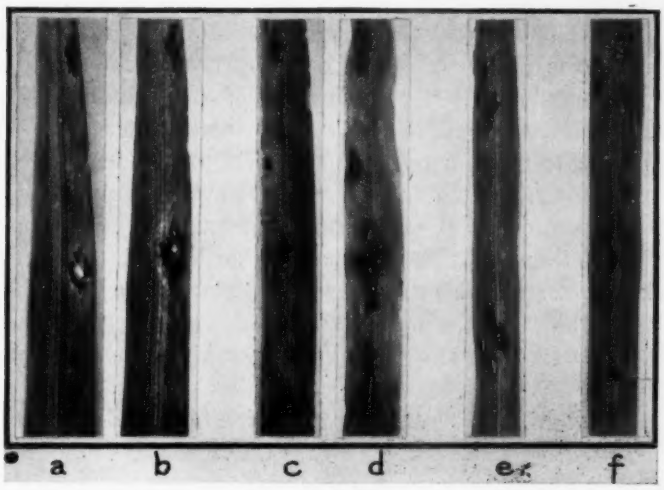


FIG. 3. a–e. Arnautka, f. Little Club. Secondary development. a. Arnautka, Exp. 34, inoculated Sept. 12, 1929, placed in dark Sept. 18, photographed Sept. 27. In dark 2 days. b. The same leaf as a, photographed Oct. 1 (4 days later). c. Arnautka, Exp. 64, inoculated Apr. 4, 1930, placed in dark Apr. 11, photographed Apr. 21. In dark 1 day. d. The same leaf as c, photographed May 2, 1930 (11 days later). e. Arnautka, Exp. 34, inoculated Sept. 12, 1929, placed in dark Sept. 18, photographed Oct. 1. In dark 4 days. f. Little Club, Exp. 66, inoculated Apr. 10, 1930, placed in dark Apr. 16, photographed Apr. 29. In dark 6 days.

or free from rust, underwent a series of changes in appearance, beginning with a slight paling that gradually increased in severity until it reached complete yellowing. This was sometimes followed by a bleaching from yellow to white. In the later stages of etiolation the leaves lost their turgor

[3] This general statement excludes the tissues in the immediate vicinity of infection centers. These come under discussion in the section on infection types.

and often took on a translucent, or injected, appearance. This was accompanied by browning and drying of the tissues. All parts of a single leaf did not progress at the same rate through this series of changes. There was a gradient from more rapid progression at the tip of the leaf to slower change at the base. Thus it happened that the first visible effect of darkness on any leaf was a slight paling of the tip. With prolonged darkness it became more chlorotic and the slight paleness progressed down the leaf. In the same way the first dead tissue appeared at the tip of the leaf and progressed downward with longer duration of darkness. The effect can be seen in figures 6, C, and 10, A,[4] where the number under each leaf represents the number of days in continuous darkness. Leaves infected by rust behaved essentially as did the noninfected ones (Figs. 5, C, 7, C, 8, A, and others).

These photographs were all taken some days after removal of the plants from the dark chamber, and the appearance of the leaves was, therefore, determined by the period of light following darkness as well as by the period of darkness itself. Those portions of the leaves that were dead when photographed were not always dead on removal from the dark. The tissues appeared to reach a point in the series of changes resulting from darkness beyond which these changes were irreversible, and they proceeded to the point of death even after return to natural light conditions. On the other hand, leaf tissue that had not reached such a point of irreversibility in the dark chamber was able to survive when placed in a favorable environment. For purposes of the present paper this crude distinction between reversibility and irreversibility of the effects of prolonged darkness will suffice.

By a comparison of the various illustrations it is evident that the rate of progression through the series of changes resulting from darkness was not the same in all experiments. The rate seemed to depend upon the wheat variety concerned, the environmental conditions during the sojourn of the plants in the dark, chiefly a matter of temperature, and the physiological state of the plants when placed in the dark, chiefly a function of age and of environmental conditions during growth. It will be observed also that in the experiment represented by figure 6, C, and others, leaves receiving the same treatment simultaneously were not always alike in their response. This sort of individual variation in behavior was frequently noted and was associated with similar heterogeneity in rust development. Certain experiments designed to discover the underlying cause for this variation will be mentioned later. A more detailed account of the dependence

[4] In figure 10, A, the white flecking at the tip of the leaf held 1 day in the dark is not a result of this treatment, for similar flecking occurred on many of the untreated leaves in this particular experiment.

of rapidity of change in appearance on environment and on the physiological state of the tissues will appear in later sections together with a discussion of its significance in relation to rust development.

Such a series of changes in appearance as has been described for wheat seedlings confined in the dark must be considered as the outwardly visible signs of a series of metabolic changes resident in the cell protoplasm. Loss of green color is consequent upon the breakdown of chlorophyll; loss of turgor upon a permeability change. Such fundamental changes could not occur as isolated processes in the complex of reactions comprising the metabolic activity of green plant tissues in the dark. The host tissues confined in the dark must, therefore, be thought of as undergoing a series of metabolic changes to the time relations of which the outwardly visible changes provide an index.

In cherry-laurel leaves changes in appearance resulting from darkness have been definitely correlated with more accurate indices of changing metabolism. According to Godwin and Bishop (12), F. F. Blackman found that yellowing of detached mature cherry-laurel leaves in the dark was associated with an increase and subsequent decrease in respiratory activity following an initial decline. Godwin and Bishop, themselves, reported that when in young leaves of the cherry laurel an increase in respiration failed to appear after the initial decline yellowing also failed, the green color giving way directly to browning and general disintegration after prolonged subjection to darkness. The same authors found a very intimate correlation between rate of yellowing, rate of loss of cyanogenetic glucosides, and respiratory intensity in cherry-laurel leaves subjected to darkness.

There is every reason to believe that in wheat leaves, also, the rate of change in appearance can be associated with the rate of progression through the sequence of physiological states characteristic of the metabolism of the tissues in continuous darkness. Whether the correlations are the same as in cherry-laurel leaves is a matter for experimental determination. Present indications from experiments in progress are that, in this respect, wheat leaves present certain dissimilarities from those of cherry laurel.

C. *Effect of continuous darkness upon the rate of rust development.* The results recorded in this section are concordant with those of other workers inasmuch as subjection of infected plants to darkness during the "incubation period"[5] of the fungus increased the time required for the development of pustules. On the other hand, the indication that others who have studied the problem have discovered that the incubation period is lengthened by a number of days equivalent to the number of days spent in the dark has not been borne out by my investigations. The actual num-

[5] In accordance with previous usage in rust literature, the term includes the period from inoculation to the appearance of pustules.

ber of days added to the incubation period by a definite sojourn of the host plants in darkness varied considerably under different circumstances, as will appear in the following account of experimental results.

It is not easy to express accurately the effect of darkness on the rate of rust development. Fromme (8), Mains (19), and Waters (31) recorded this effect in terms of number of days of retardation in the development of uredinia, and a similar system has been adopted here. In my experiments, however, the experimental treatment caused not only retardation but also inhibition of pustule development. Pustules did not always form in infection flecks and, when they did form, did not always open, nor did they always reach their full size. Lengthening of the incubation period was but one of the effects of darkness.

The length of the period of time from the first appearance of infection flecks to the point where most of these bore pustules as brownish swellings under the epidermis was selected as an index to the rate of development of the rust. The compilation of daily notes upon the appearance of infected leaves made possible a comparison of the progress of the rust at various periods of development, and it turned out that prolongation of the incubation period was accomplished chiefly in the period selected. The earlier part of the incubation period was not affected, for it was only at its close, when infection flecks first appeared, that the plants were placed in the dark chamber. The interval during which pustules were breaking through the epidermis was sometimes affected by a period of darkness, but it did not necessarily increase in duration. On the contrary, it was sometimes curtailed after considerable periods in the dark. The explanation of this is that when pustules were formed on plants left long in the dark they often opened while still very much smaller than nonerumpent pustules on susceptible control plants.

The duration of this intermediate period of rust development having been selected as the best single numerical index of the rate of development of the rust, the information derived from all of my experiments concerning the effect of continuous darkness upon this period is presented in table 1.

The number of days between the appearance of infection flecks and the development of pustules in most of these, under different treatments, was determined by a scrutiny of my daily greenhouse records, showing the proportion of open to closed pustules and of infection flecks with pustules to those without. The proportion was estimated after each leaf had been examined carefully and the number of leaves bearing open pustules, those bearing closed pustules, and the number bearing infection flecks without pustules were taken into account. If, for any reason, it was impossible to tell with reasonable certainty from my records on which of two days the

TABLE 1.—*A summary of experimental results concerning the effect of continuous darkness upon the rate of pustule development of Puccinia graminis tritici p. f. 21 on 11 differential host varieties*

| Variety | Exp. no. | Date of inoculation | Time from inoc. to appearance of infection flecks Days | Lapse of time in days between the first appearance of infection flecks and the stage when most of these had formed pustules, after subjection to darkness for: | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0 day | 1 day | 2 days | 3 days | 4 days | 5 days | 6 days | 7 days |
| I[f] | II | III | IV | V | VI | VII | VIII | IX | X | XI | XII |
| Little Club | 10a | Apr. 10, '29 | 7 | 2–3 | 4–5 | 5–6 | 7–8 | .....[b] | ..... | ..... | ..... |
| | 12a | " 20, " | 7 | 2 | 3 | 7–8 | (9)[c] | (9)[c] | | | |
| | 14c | " 23, " | 8 | 3 | ..... | 6–7 | (8) | (9) | ..... | ..... | ..... |
| | 15c | May 3, " | 7 | 3 | 3 | ..... | (7–8) | (9) | (9–10) | ..... | ..... |
| | 20 | " 22, " | 6 | 2 | 4 | 7–8 | (13–15) | (13–15) | (15–16) | –[d] | ..... |
| | 24 | June 17, " | 5 | 1 | 3 | 4–5 | 5 | 5 | 10 | – | ..... |
| | 27 | July 12, " | 5 | 1 | 2 | 5–6 | 6 | 7–8 | – | – | ..... |
| | 30 | " 24, " | 4 | 3 | 4–5 | 9–10 | (11–12) | – | – | – | ..... |
| | 41 | Oct. 17, " | 6 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| | 42u | " 20, " | 6 | 2 | ..... | ..... | 5–6 | (7) | (7) | (7–8) | (8–9) |
| | 43 | " 23, " | 6 | 4 | ..... | 7 | 9 | 10 | >12 | >15 | ..... |
| | 49 | Nov. 23, " | 7 | 4 | ..... | ..... | 8 | 8 | ..... | (12–13) | ..... |
| | 50a | " 29, " | 9 | 3 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| | 56c | Jan. 2, '30 | 7[a] | 2 | ..... | 6 | 6 | 8 | (10) | (11) | (13) |
| | 56b | " 2, " | 7[a] | 2 | ..... | 5–6 | 6 | 8 | (11) | (11) | (12) |
| | 56a | " 2, " | 7[a] | 2 | ..... | 6 | 6–7 | 8 | (10) | (11) | (12) |
| | 63 | Mar. 29, " | 7 | 3 | 4 | 5 | 6 | 8 | 10 | (11–12) | ..... |
| | 65 | Apr. 8, " | 6 | 2 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| | 66 | " 10, " | 6 | 3 | 4 | 7–8 | (13) | (13) | (11–12) | – | ..... |
| | 71c | " 26, " | 5 | 2 | 4 | 4 | 6 | 7 | (7) | – | ..... |
| | 74 | " 19, " | 8 | 2–3 | 3–4 | 5 | 8 | (8) | (8) | (12–13) | ..... |
| Marquis | 6 | Mar. 15, '29 | 6 | 2 | 4 | 8 | 9 | ..... | ..... | ..... | ..... |
| | 7 | " 28, " | 8 | 2 | 3–4 | 6–7 | 8–9 | ..... | ..... | ..... | ..... |
| | 10b | Apr. 10, " | 7 | 2–3 | 4–5 | 5–6 | 7–8 | ..... | ..... | ..... | ..... |
| | 21a | May 29, " | 6 | 2 | ..... | 6–7 | 8–9 | – | – | ..... | ..... |
| | 21b | " 29, " | 6 | 2 | ..... | 6–7 | 8–9 | – | – | ..... | ..... |
| | 67 | Apr. 4, '30 | 7 | 3 | 4 | 6 | 7 | (5) | – or (11) | – | ..... |

TABLE 1.—*Continued*

| Variety | Exp. no. | Date of inoculation | Time from inoc. to appearance of infection flecks Days | Lapse of time in days between the first appearance of infection flecks and the stage when most of these had formed pustules, after subjection to darkness for: | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0 day | 1 day | 2 days | 3 days | 4 days | 5 days | 6 days | 7 days |
| I | II | III | IV | V | VI | VII | VIII | IX | X | XI | XII |
| Kota | 12b | Apr. 20, '29 | 5 | 4–5 | 6 | 7–8 | 9–10 | (11–13) | ..... | ..... | ..... |
| | 19 | May 16, " | 6 | 4 | 5–6 | 6–8 | 8–9 | 8–9 | ..... | ..... | ..... |
| | 29 | July 20, " | 4 | 3 | 4 | 7 | – | – | – | – | ..... |
| | 39 | Oct. 7, " | 6 | 4–5 | 5–6 | 6–8 | ..... | – | – | – | ..... |
| | 62 | Mar. 20, '30 | 8 | 2 | 4 | 6–7 | 10–11 | (9–10) | – | – | ..... |
| | 79 | May 24, " | 6 | 3 | 5 | 7 | 8 | – | – | – | ..... |
| Arnautka | 17b | May 11, '29 | 6 | 2 | 4 | 7 | (9) | (11) | – | ..... | ..... |
| | 28 | July 16, " | 5 | 2 | 3–4 | 7 | (12) | – | – | ..... | ..... |
| | 31 | Aug. 2, " | 6 | 2 | 3 | 4–5 | (5–7) | – | – | – | ..... |
| | 34 | Sept. 12, " | 6–7 | 3–4 | 5–6 | 7–8 | (8–9) | (11–12) | (12–13) | ..... | ..... |
| | 36 | " 28, " | 7 | 3 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| | 37 | " 29, " | 7 | 3 | ..... | 6–7 | (7–8) | – | – | ..... | ..... |
| | 58 | Jan. 13, '30 | 7 | 2 | 4 | ..... | 9 | ..... | ..... | – | – |
| | 64a | Apr. 4, " | 7 | 2 | 5 | 6 | – | – | – | – | ..... |
| | 64b | " 4, " | 7 | 2 | 5 | 6 | – | – | – | – | ..... |
| Mindum | 10e | Apr. 10, '29 | 7 | 2 | 4 | 6–7 | 5 or more | ..... | ..... | ..... | ..... |
| | 16b | May 8, " | 6 | ..... | ..... | ..... | ..... | – | – | ..... | ..... |
| | 17a | " 11, " | 7 | 1 | 3 | ..... | 4–5 | ..... | (10) | ..... | ..... |
| | 77 | " 11, '30 | 7 | 3 | 4 | (6) | ..... | – | – | – | ..... |
| Speltz Marz | 15b | May 3, '29 | 7 | 3 | 4 | 7 | 8 | (9–10) | ..... | ..... | ..... |
| | 73 | Apr. 27, '30 | 6 | 1–2 | 4 | 5 | 5 | 7–8 | (7) | (7–8) | ..... |
| | 76 | May 11, " | 7 | 2–3 | 4 | 6–7 | – | – | – | or – | ..... |
| Kubanka | 75 | May 8, " | 7 | 3 | 5 | (7) | – | – | – | – | ..... |
| | 78 | " 24, " | 6 | 2 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |

TABLE 1.—*Continued*

| Variety | Exp. no. | Date of inoculation | Time from inoc. to appearance of infection flecks Days | Lapse of time in days between the first appearance of infection flecks and the stage when most of these had formed pustules, after subjection to darkness for: | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0 day | 1 day | 2 days | 3 days | 4 days | 5 days | 6 days | 7 days |
| I[f] | II | III | IV | V | VI | VII | VIII | IX | X | XI | XII |
| Acme | 16a | May 8, '29 | 7 | 1–2 | 3–4 | 4–5 | (>15) | – | – | ..... | ..... |
| | 23 | June 12, " | ..... | 1 | 1–2 | 2–4 | – | – | – | ..... | ..... |
| | 26 | July 6, " | 5 | 2 | 4–5 | 4–5 | 9 | – | – | ..... | ..... |
| | 32 | Aug. 9, " | 5 | 3 | 3–4 | ..... | ..... | – | ..... | ..... | ..... |
| | 40 | Oct. 11, " | 6 | 1–3 | 5 | 7 | (7–8) | – | – | – | ..... |
| | 65 | Apr. 8, '30 | 6 | 2–3 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Einkorn | 14b | Apr. 23, '29 | ..... | (9) | (9) | (11–12) | – | ..... | ..... | ..... | ..... |
| | 38 | Sept. 30, " | 7 | 8 or more | (6–8) | 8 or more | – | – | – | – | ..... |
| | 65 | Apr. 7, '30 | 6 | 3–6[e] | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| | 72 | " 26, " | 5 | 3 | (6–8) | (11) | – | – | – | – | ..... |
| Vernal | 15a | May 3, '29 | 5 | 8 | 8 | (11) | (12–13) | (14) | ..... | ..... | ..... |
| | 65 | Apr. 7, '30 | 6 | 6–7 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| Khapli | 12c | Apr. 20, '29 | 6 | 6 | 10 | (13) | – | – | ..... | ..... | ..... |
| | 22 | June 3, " | 7 | 2–3 | (>9) | – | – | – | – | ..... | ..... |
| | 35 | Sept. 14, " | 8 | 4 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| | 59 | Jan. 13, " | 7 | 5 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| | 65 | Apr. 7, '30 | ..... | 3–5 | ..... | ..... | ..... | ..... | ..... | ..... | ..... |

[a] Continuous artificial light was added to daylight.
[b] A series of dots (.....) signifies that the data required to fill a certain space were lacking.
[c] Brackets around a number signify that all of the infection flecks did not produce pustules.
[d] A minus sign (–) signifies that few or none of the infection flecks developed pustules as long as the plants were kept under observation or until they died.
[e] One pot required 3 days, 1 pot required 6 days.
[f] The Roman numerals at the heads of columns are introduced merely for convenience in referring to the contents of the table.

end of a development period had been reached, the possible alternatives for the duration of the period were recorded (*e.g.*, 2–3, 4–5). For a few experiments, open pustules, closed pustules, and flecks without pustules were actually counted.

Table 1, column IV gives the duration of the first period in rust development, the time from inoculation until the first appearance of infection flecks. This column is included to indicate the rate of development of the fungus previous to subjection to darkness in the various experiments. The addition of the values in this column to the values for corresponding experiments in columns V–XII will give values that represent the incubation periods after the various treatments, for they represent the time from inoculation until most of the pustules have become visible and a few of them have ruptured the epidermis. Columns V–XII deal directly with the duration of the period of pustule formation. Column V shows the number of days required on control plants allowed to develop undisturbed in the greenhouse. Columns VI–XII show the time required for pustule formation after subjection of the plants to darkness for 1 to 7 days, beginning with the inception of the period of pustule development.

It is obvious from a study of this table that the treatment caused a prolongation of this period but that the prolongation was not always equivalent to the period of darkness. For instance, 1 day in the dark might retard pustule development either not at all or by 1 day or by more than 1 day; even a 6-day retardation was once recorded for Khapli. The general tendency was for pustule development to be retarded a longer time than that spent in the dark by the infected host plants.

The possible confusion of secondary with delayed primary development (p. 522) presents a source of error in recording retardation in rust development. If, however, this error affects the records, it masks rather than accentuates the discrepancy between the duration of darkness and the retardation in primary rust development. This discrepancy may, therefore, be even greater than it appears to be in these records.

*Recapitulation.* In every experiment darkness delayed pustule formation, the period of delay being roughly proportional but not necessarily equivalent to the time in darkness. The numerical index chosen was regarded as the best medium available for expression of the effect of my experimental treatment on the rate of rust development. If duration of the incubation periods had been presented, it would have shown practically the same discrepancy between the dark period and prolongation of the incubation period. It is not intended that precise significance should be attached to these numerical values. The fact that secondary development could not always be distinguished from primary made difficult precise measurement of rate of development, as did also the fact that primary rust development was

often not merely retarded but modified and inhibited (p. 510). Rates of development in different experiments are not comparable because of insufficient standardization in the age of the seedlings employed and because of differences in environmental conditions. Rates of development in any one experiment, however, are comparable, and, in spite of the variety of experimental conditions introduced, in no case were these so adjusted as to cause equivalence between retardation and duration of darkness in all of the periods of darkness involved in one experiment. Isolated instances of equivalence occurred, but so infrequently as to make them seem of no general significance.

These observations differ from those of Fromme (8) and Mains (19), who, in a few experiments with *Puccinia coronata* on oat seedlings, discovered indications that subjection to darkness after inoculation caused a period of retardation in pustule development equal to the period of darkness. Obviously, such indications do not find substantiation under the conditions of my more numerous experiments with *P. graminis tritici* on wheat seedlings; nor does the theory based upon the equivalence of the two periods in question, namely, that during the time the host is in the dark the development of the fungus is arrested because of cessation of carbon assimilation in the host tissues, and on return to light, proceeds at its previous pace. Mains (19) cited one instance where retardation in rust development exceeded the 11 days of darkness. This he treated as an exceptional case, suggesting that the extremely long dark period had disarranged the physiological processes of the host so that on its return to light these were carried on poorly and were, therefore, unable to furnish the fungus enough food to allow it to resume growth immediately at its previous rate. A discrepancy between retardation and the duration of darkness, treated as exceptional by Mains, proved very general in my experiments. His explanation of the single case in which retardation exceeded the dark period does not adequately account for the results of my experiments. As already pointed out (p. 503), host physiology is regarded as entering upon an altered course as soon as the plants are transferred to darkness, and, whether the dark period be long or short, the physiologic state of the tissues, not only during the light period following darkness but also during the dark period itself, is considered important in determining the degree of retardation in rust development. Furthermore, there is evidence that rust development in my experiments was not completely arrested during darkness. Minute pustules have been seen to develop and break open while the host was in the dark chamber. The following pages will bring additional evidence that during the sojourn of the host in darkness development of the rust was not simply arrested but its relation to the host was strongly modified and its development retarded, presumably because of the modified host relations resulting from darkness.

D. *Effect of continuous darkness on infection type.* Figure 2, representing infection types of wheat stem rust as defined by Stakman and Levine (25), represents some standard reactions of wheat varieties to rust forms and is a composite photograph partly of control leaves and partly of leaves held in darkness for different periods of time. It includes 5 varieties of wheat infected by *Puccinia graminis tritici* p. f. 21. (See explanation of plate.) The ordinary reactions of these 5 varieties to form 21 are presented in figure 1. It will be observed that the one variety, Arnautka, infected with the single rust form, appears in figure 2 as a representative of every type of infection. Sufficient continuous sojourn of infected wheat seedlings in the dark at the time when pustule development is in progress has evidently resulted in a modification of those symptoms that indicate the degree of compatibility between host and pathogen and are the basis for expressing the standard reactions of the differential hosts to physiologic forms of *P. graminis tritici.* About 70 experiments with seedlings subjected to periods of continuous darkness have had this result in common.

Figures 4–9 illustrate the outcome of several experiments. Photographic records of nearly all the experiments were obtained, but it is, of course, impossible to present them all here. Those have been selected that illustrate best the nature of my experimental results. Each photograph includes leaves that represent the results of one experiment. In figure 4, A, for example, the first leaf on the left was selected as representative of plants allowed to develop naturally; the next leaf was one of those deprived of light for 1 day; and the rest, those deprived of light 2, 3, 4, and 5 days. The progression is always from untreated leaves, at the left, to those longest in darkness, at the right. For convenience in relating a photograph to its proper experimental treatment, a number is printed below each leaf to indicate the number of days of darkness to which the leaf was subjected.

One leaf in the photographs represents 2, or sometimes 3, pots, *i.e.*, 15 to 40 1-leaf plants. It was often difficult to choose a single leaf entirely representative of all the plants in the lot, for there was, as with effects on the general appearance of the host tissues, a certain amount of individual variation amongst the plants in regard to the effect of any period of darkness on the infection type. Where the variation was too great, more than one leaf was included in the illustration.

In one of the photographs (Fig. 8, A) a noninoculated leaf is to be seen beside each rusted leaf. Photographs of noninoculated controls were made for many of the experiments, but for the most part separately from those of inoculated leaves. Most of these are omitted, and reference is made to noninoculated controls by description only.

Most of the photographs were taken when the rust on control plants had reached full development, that is, about 15 to 20 days after inoculation.

FIG. 4. A. Khapli, Exp. 22, inoculated June 3, 1929, placed in dark June 10, photographed June 20. Left to right: 1 leaf untreated, 1 leaf in dark 1 day, 2 leaves in dark 2 days, 1 leaf each in dark 3 days, 4 days, 5 days. a. Minute pustules on a leaf in dark 4 days. B. Acme, Exp. 16a, inoculated May 8, 1929, placed in dark May 15, photographed May 30. Left to right: 1 leaf untreated, 2 leaves in dark 1 day, 1 leaf in dark 2 days, 2 leaves in dark 3 days, 1 leaf in dark 4 days. a. Minute pustules in green islands. b. Minute necrotic flecks without pustules. c. Minute pustules on leaves in dark 1 day. d. Green areas marking infection centers. C. Speltz Marz, Exp. 15b, inoculated May 3, 1929, placed in dark May 10, photographed May 21. Left to right; 1 leaf each: untreated, in dark 1 day, 2 days, 3 days, 4 days.

This was considered the best time to illustrate the effect of darkness by a single photograph, although no single photograph can be entirely adequate because of the progressive nature of rust development under the influence of the period of darkness imposed and the period of natural illumination following it (p. 524).

A complete set of experiments on 11 differential hosts was carried out during the spring and early summer of 1929, and several of the varieties were used in the same season of 1930. One experiment on each variety is described in this section.

KHAPLI, EXPERIMENT 22 (Fig. 4, A)—24 pots planted May 27, 1929. 12 pots inoculated June 3. To dark chamber June 10. Photographed June 20, 17 days after inoculation, 4 days after return of "5-day" plants to light. (The "4-day" leaves had, therefore, been in the light 5 days after their sojourn in the dark, the "3-day" leaves 6 days, etc.[6]

*Reaction of controls*—"1".

*Effect on infection type of continuous darkness for: One day*—some flecks without pustules. *Two days*—reaction "1=", *i.e.*, flecks small, very few with pustules. *Three, four days*—further decrease in size of flecks and number of pustules. (The pustules on the 4-day leaves photographed are so minute as to be difficult of discovery but are present in Fig. 4, A). *Five days*—no pustules, no necrosis, indefinite nonnecrotic flecks with green rings,[7] as in Fig. 4, A.

ACME, EXPERIMENT 16a (Fig. 4, B)—12 pots planted April 26, 1929. Inoculated May 8. To dark chamber May 15. Photographed May 30, 22 days after inoculation, 11 days after return of the 4-day leaves to light.

*Reaction of controls*—"4c".

*Effect on infection type of continuous darkness for: One day*—small pustules with large ones. (At the time of photographing, 22 days after inoculation, the small pustules were pretty well hidden, but a few can be picked out at 4, B, c. Such small pustules may occur at times in "4" reactions, but in this experiment the 1-day plants could be distinguished from the control plants by the presence of these minute pustules).

*Two days*—"x" reaction, *i.e.*, greater proportion of small pustules, some in necrotic, some in nonnecrotic flecks. (It will be observed that in the leaf photographed most

[6] The length of time the leaves shown in any of the figures had already been exposed to normal light conditions after removal from the dark chamber can always be computed from the information given in the legends accompanying the figures. The reader may be assured that, although it was impossible to avoid a discrepancy with regard to the times the various leaves in a figure had been allowed to recover, the effects of this discrepancy were small as compared with the actual effects of the different periods of darkness.

[7] "Green rings" are distinguished from "green islands." The latter term implies, in this paper, a hypersensitive halo (Fig. 2 c, d); the former, a ring of tissue around a rust infection that remained a deeper green than the surrounding tissue after subjection to darkness (Fig. 8, C, base of 5-day leaf). Such rings always appeared on plants left in the dark long enough to cause paling. Green rings may be narrow or broad and grade into "green areas."

of the large pustules present are toward the base of the infected area. Toward the upper end at 4, B, a, are a few pustules in small green islands, surrounded by necrotic halos. It was frequently observed with other varieties, as well as with Acme, that, especially in leaves held for shorter times in the dark, the more "susceptible" types of pustules predominated toward the base of a leaf, while the more "resistant" types predominated toward the tip (Figs. 4, B and C, 7, A). This distribution of types is associated with the physiological gradient already mentioned (p. 502) and at all times apparent in the leaves of noninoculated as well as inoculated seedlings kept in the dark.) *Three days*—"x" reaction, *i.e.*, a few large pustules intermingled with resistant types strikingly like Khapli, although necrosis not so extensive as that usually resulting from infection of Khapli; a few minute necrotic flecks, as at b, developed very early. (The large pustules accomplished their entire development during the 11 days that elapsed between returning the leaves to natural light conditions and photographing them.) *Four days*—only 1 pustule at any time; this appeared 8 days after return to light and 10 days later had reached normal size; all other infections minute; indistinct, nonnecrotic flecks in dark green areas on paler leaf, *e.g.*, at d in figure 4, B; only two leaves survived until photographed. *Five days*—survived only 3 days after return to light, no pustules, green areas on pale leaves, as 4-day leaves.

VERNAL, EXPERIMENT 15a (Fig. 5, A)—9 pots planted April 22, 1929. Inoculated May 3. To dark chamber May 8. Photographed May 21, 18 days after inoculation and 9 days after return of 4-day leaves to light.

*Reaction of controls*—"1".

*Effect on infection type of continuous darkness for: One day*—little change except in rate of development. *Two days*—smaller pustules. *Three days*—no pustules until 14 days after inoculation (6 days after return to light), then fairly numerous pustules, as in figure 1. *Four days*—one pustule on 7th day after return to light, then fairly numerous pustules.

SPELTZ MARZ, EXPERIMENT 15b (Fig. 4, C)—9 pots planted April 22, 1929. Inoculated May 3. To dark chamber May 10. Photographed May 21, 18 days after inoculation and 7 days after return of 4-day leaves to light.

*Reaction of controls*—"4".

*Effect on infection type of continuous darkness for: One day*—no change. *Two days*—almost no change, development slower. *Three days*—"x" reaction. *Four days*—very few large pustules, practically all small with nonnecrotic flecks, a few green islands.

MINDUM, EXPERIMENT 17a (Fig. 5, B)—12 pots planted April 30, 1929. Inoculated May 11 (4 in. high or more). To dark chamber May 18. Photographed May 29, 18 days after inoculation and 6 days after return of the 5-day leaves to light.

*Reaction of controls*—"4" (not quite completely developed when photographed).

*Effect on infection type of continuous darkness for: One day*—a little hypersensitive tissue,[8] most pustules soon developed to "4" type. *Two, three, four, five days*—

[8] The term "hypersensitive tissue" is used in a descriptive sense to indicate the visible result of a definite incompatibility between host and pathogen. It includes necrotic tissue and such nonnecrotic tissue as is sufficiently well defined to be distinct from the more diffuse "chlorotic tissue."

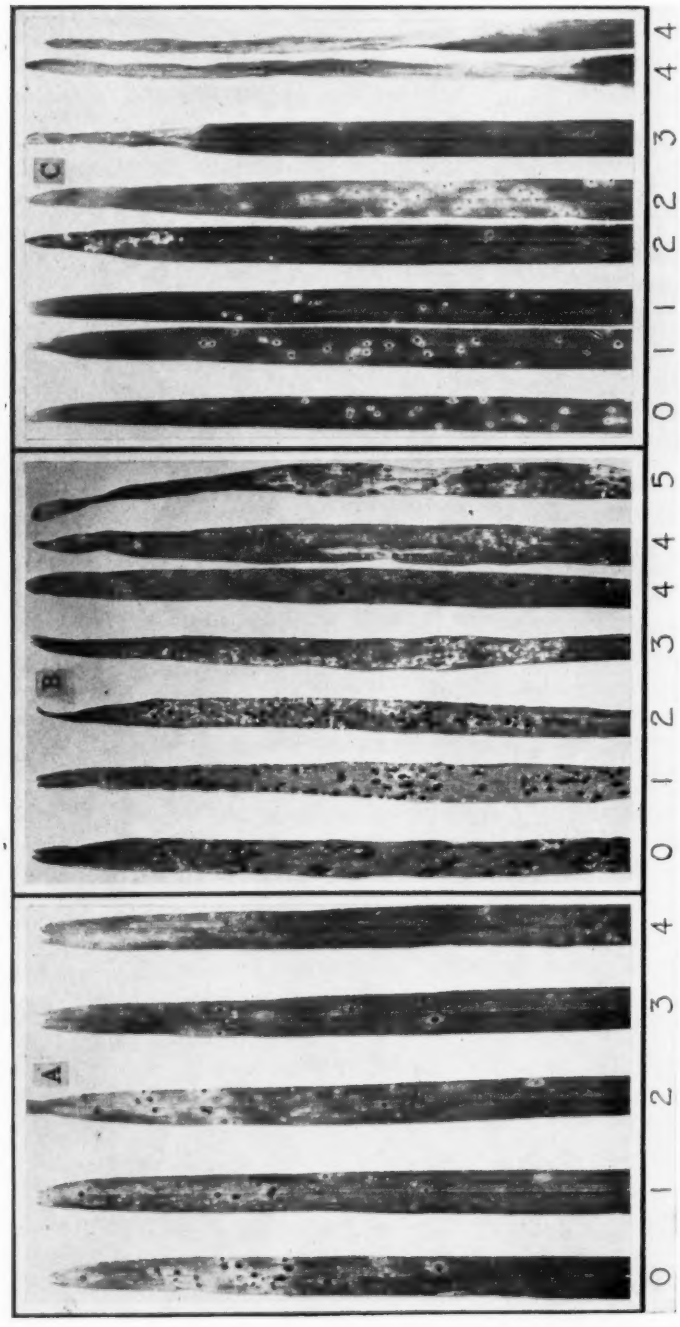


FIG. 5. A. Vernal, Exp. 15a, inoculated May 3, 1929, placed in dark May 8, photographed May 21. Left to right, 1 leaf each: Untreated, in dark 1 day, 2 days, 3 days, 4 days. B. Mindum, Exp. 17a, inoculated May 11, 1929, placed in dark May 18, photographed May 29. Left to right, 1 leaf each: untreated, in dark 1 day, 2 days, 3 days; 2 leaves in dark 4 days; 1 leaf in dark 5 days. C. Einkorn, Exp. 72, inoculated Apr. 26, 1930, placed in dark May 1, photographed May 7. Left to right: 1 leaf untreated, 2 leaves in dark 1 day, 2 leaves in dark 2 days, 1 leaf in dark 3 days, 2 leaves in dark 4 days.

greater proportion of hypersensitive tissue. Response similar to Arnautka, experiment 17b.

KUBANKA, EXPERIMENT 14a (no illustration)—9 pots planted April 13, 1929. Inoculated April 23. To dark chamber April 28.

*Reaction of controls*—"3" reaction as a result of very heavy infection.

*Effect on infection type of continuous darkness for: One, two days*—a little hypersensitive tissue. *Three days*—"x"—(variation from minute pustules in nonnecrotic flecks to normal pustules); certain areas almost entirely etiolated, with very minute pustules. *Four days*—resembled 3-day leaves, but the symptoms of incompatibility recorded as more pronounced.

ARNAUTKA, EXPERIMENT 17b (Fig. 7, A)—12 pots planted April 30, 1929. Inoculated May 11 (4 in. high or more). To dark chamber May 17. Photographed June 3, 23 days after inoculation and 12 days after return of 5-day plants to light.

*Reaction of controls*—"4".

*Effect on infection type of continuous darkness for: One day*—no change in reaction, development slower. *Two days*—tiny pustules in nonnecrotic flecks among large ones. *Three, four, five days*—proportion of "resistant"[9] to "susceptible" pustules increased. As in the other varieties, the susceptible pustules were always later in developing than the resistant ones.

EINKORN, EXPERIMENT 72 (Fig. 5, C)—21 pots planted April 16, 1930. Fourteen pots inoculated April 26 (3–3¾ in. high). To dark chamber May 1. Photographed May 7, 11 days after inoculation, 2 days after return of the 4-day leaves to light.

*Reaction of controls*—"1".

*Effect on infection of continuous darkness for: One day*—unchanged except in rate of development. *Two days*—more diffuse hypersensitive areas (as shown in one 2-day leaf in figure 5, C). In the center of the indefinite yellow area a sharply defined translucent area usually appeared. In this area a pustule sometimes developed. *Three days*—flecks, for the most part minute, necrotic or white and nonnecrotic, or merely green specks on a paler background visible only by transmitted light. Two days after photograph for figure 5, C, was taken and 5 days after the period of darkness was completed, the 3-day leaves produced 2 minute pustules. Still later, pustules appeared in several flecks but never in so many as ½ of them, whereas on control leaves, pustules appeared in ⅔ of the flecks. *Four days*—no pustules at any time, very few flecks. Many leaves died within 2 or 3 days after their return to light. *Five, six days*—dead on removal from the dark chamber.

MARQUIS, EXPERIMENT 21a (Fig. 6, B)—20 pots planted May 21, 1929. Ten pots inoculated May 29 (2–3 in. high). To dark chamber June 4. Photographed June 11, 13 days after inoculation and 2 days after return of the 5-day leaves to light.

*Reaction of controls*—"4–c" (not fully developed when photographed).

*Effect on infection type of continuous darkness for: Two days*—when photographed tiny pustules and definitely chlorotic areas, later developed type-"3" infection. *Three days*

[9] The terms "resistant" and "susceptible" are used in a descriptive sense to indicate the sort of infection centers that go to make up a resistant or susceptible infection type as defined by Stakman and Levine (25).

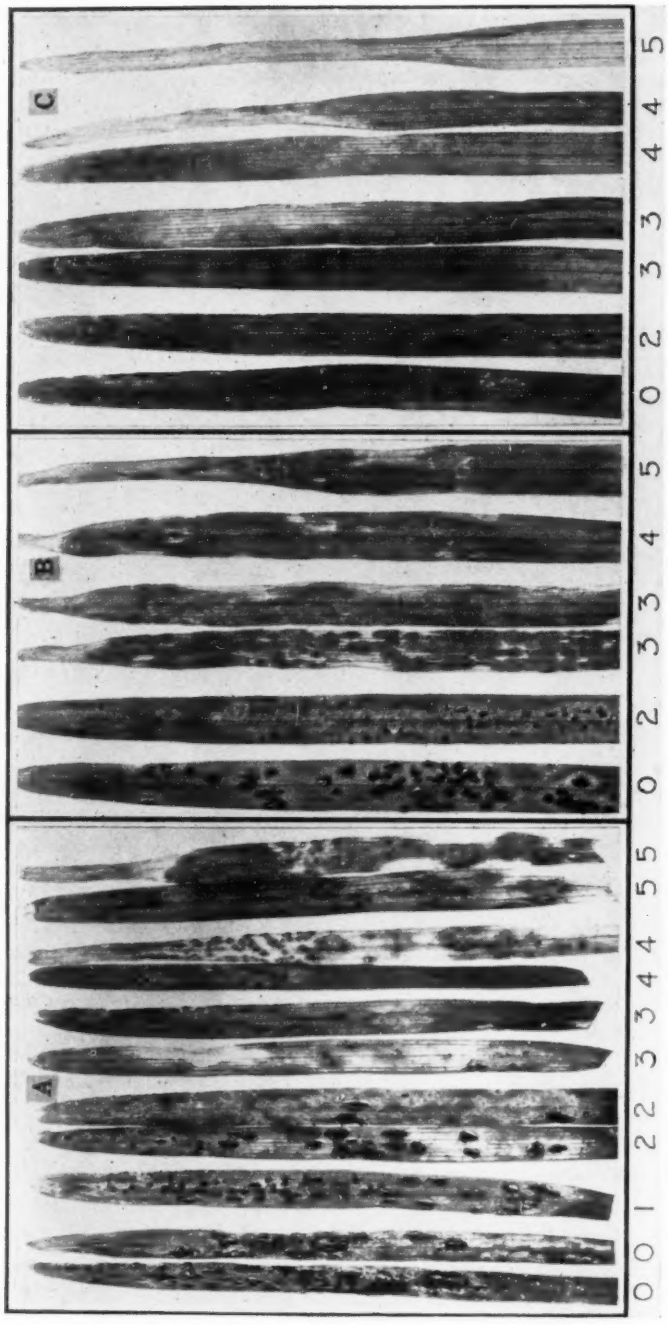


FIG. 6. A. Little Club, Exp. 20, inoculated May 22, 1929, placed in dark May 28, photographed June 10. Left to right: 2 leaves untreated, 1 leaf in dark 1 day, 2 leaves in dark 2 days, 2 leaves in dark 3 days, 2 leaves in dark 4 days, 2 leaves in dark 5 days. B. Marquis, Exp. 21a, inoculated May 29, 1929, placed in dark June 4, photographed June 11. Left to right: 1 leaf untreated, 1 leaf in dark 2 days, 2 leaves in dark 3 days, 1 leaf in dark 4 days, 1 leaf in dark 5 days. C. Marquis, Exp. 21a, noninoculated, placed in dark June 4, photographed June 11. Left to right: 1 leaf untreated, 1 leaf in dark 2 days, 2 leaves in dark 3 days, 2 leaves in dark 4 days, 1 leaf in dark 5 days.

—green islands, particularly striking on some leaves. Minute pustules had developed in some of the green islands when the photograph was taken. Three days later these were as large as "3" pustules, but a certain amount of hypersensitive tissue remained visible. *Four days*—green islands, further suppression of pustules. *Five days*—no pustules, indefinite nonnecrotic flecks with green islands or rings.

KOTA, EXPERIMENT 19 (Fig. 8, A)—20 pots planted May 6, 1929. Ten pots inoculated May 16. To dark chamber May 22. Photographed June 1, 16 days after inoculation and 6 days after return to the 4-day leaves to light.

*Reaction of controls*—"3++c" or "4=c".

*Effect on infection type of continuous darkness for: One day*—no change in reaction. *Two days*—green islands with necrotic rings that, in areas of crowded infection, appeared as irregular necrotic patches, sometimes forming a network. *Three days*—more necrosis, smaller pustules. *Four days*—about as the 3-day leaves.

LITTLE CLUB, EXPERIMENT 20 (Fig. 6, A)—20 pots planted May 14, 1929. Twelve pots inoculated May 22 (2–3 in. high). To dark chamber May 28. Photographed June 10, 19 days after inoculation and 7 days after return of the 6-day leaves to light.

*Reaction of controls*—"4++". (White speckling on controls distinct from the flecking resulting from rust infection on darkened leaves).

*Effect on infection type of continuous darkness for: One day*—little change. (Notice slight hypersensitiveness at the tip of the 1-day leaf in figure 6, A). *Two days*—tiny pustules in nonnecrotic flecks, mingled with large pustules. The proportion of susceptible to resistant pustules on 11 leaves varied within the limits shown by the 2 "2-day" leaves in figure 6, A. These 11 leaves, therefore, were designated as "x" to "x+". Four "4" leaves also present. *Three days*—a range from "0"; to "x–" on 14 leaves. *Four days*—chiefly very small green islands with minute brownish nonnecrotic flecks; few pustules; no large pustules up to 22 days after inoculation. (Notice the 2 very sharply defined flecks toward the upper end of 1 of the 4-day leaves illustrated). *Five days*—as 4-day leaves. *Six days*—leaves killed without producing more than green blotches on very pale leaves, a few developing green islands with minute flecks. Dead when the photograph for figure 6, A was taken.

*Recapitulation.* The experiments just described show that the interposition of a more or less extended period of darkness at the beginning of pustule formation resulted in a loss of compatibility between the fungus and the host cells. This disadjustment was in all varieties made manifest in a pronounced modification of the infection type as read at a time when the rust on control plants had reached full development. The infection types induced on susceptible varieties were found to have all the characters ordinarily associated with standard infections of *Puccinia graminis tritici* on seedling hosts of various degrees of resistance. The accepted insignia of resistance thus appeared upon every tested variety ordinarily susceptible to *P. graminis tritici* p. f. 21 and were intensified on hosts ordinarily resistant. Host varieties that usually yield a type-"4" infection with this form were induced to bear small pustules of type-"3," green islands

which characterize the standard "2" reaction, minute pustules in flecks, sometimes sharply necrotic, which distinguish the type-"1" infection, or no pustules at all. Since type-"4" pustules were sometimes formed together with pustules of the resistant types, the resulting appearance at the time of photographing was frequently that of an "x" reaction. On host varieties in which the standard infection types are resistant, the size of pustules and of flecks was reduced with increasing duration of darkness till finally definite flecks were no longer formed.

It was clear from these experiments not only that the experimental treatment induced modification of infection type on susceptible wheat varieties to resemble that on resistant varieties but also that the degree of modification depended in any one experiment upon the duration of the period of darkness. The first modification of infection type was often in the nature of a reduction in size of pustule only. When hypersensitive tissue first appeared it generally affected only a small proportion of the infection centers and, after increasing periods of darkness, affected an increasingly large proportion of the infections on a leaf. In all experiments a period of darkness was found beyond which the proportion of infection centers accompanied by hypersensitive tissue was not increased with longer duration of darkness. In some experiments the maximum proportion was not reached until after comparatively long dark periods (Fig. 7, B, where hypersensitive tissue is shown to have increased with increasing darkness up to a 5-day period).[10] In some experiments the maximum was reached after a shorter dark period (Figs. 4, B; 6, B; 7, A; 8, A). In still other experiments, necrotic tissue appeared in association with numerous infection centers after 1 day in the dark and in no larger proportion after longer periods of darkness (Fig. 8, B). It was sometimes true of experiments on the durum varieties that 1 day in the dark was sufficient to cause the appearance of minute necrotic flecks in most of the infection centers and that longer dark periods did not increase the proportion of infection centers that were represented by flecks. This was true, for instance, of the experiment represented by figure 9, A, although secondary development of the rust (see below) had hidden the flecks on the 1-day leaves when this photograph was taken. If darkness were sufficiently prolonged in any experiment beyond the time required to produce the maximum of necrosis, the amount of necrosis might again be diminished or finally fail to appear (Fig. 4, B, 4-day leaf, or Fig. 7, B, 6-day leaf).[11] This failure of necrosis on the plants held longest in the dark resembled the effect of darkening upon resistant varie-

[10] The general necrosis at the tip and base of the 6-day leaf was distinct from that representing hypersensitive tissue. It resulted from darkening and could be seen on noninfected as well as infected leaves. In this case, only areas infected by rust remained green and turgid.

[11] Cf. footnote 10.

FIG. 7. A. Arnautka, Exp. 17b, inoculated May 11, 1929, placed in dark May 17, photographed June 3. Left to right: 1 leaf untreated, 1 leaf in dark 1 day, 1 leaf 2 days, 2 leaves 3 days, 2 leaves 4 days, 2 leaves 5 days. B. Kota, Exp. 62, inoculated Mar. 20, 1930, placed in dark Mar. 28, photographed Apr. 5. Left to right: 1 leaf untreated, 1 leaf each in dark 1 day, 2 days, 3 days, 4 days, 5 days, 6 days. C. Kota, Exp. 79, inoculated May 24, 1930, placed in dark May 30, photographed June 12. Left to right, 2 leaves each: untreated, in dark 1 day, 2 days, 3 days, 4 days.

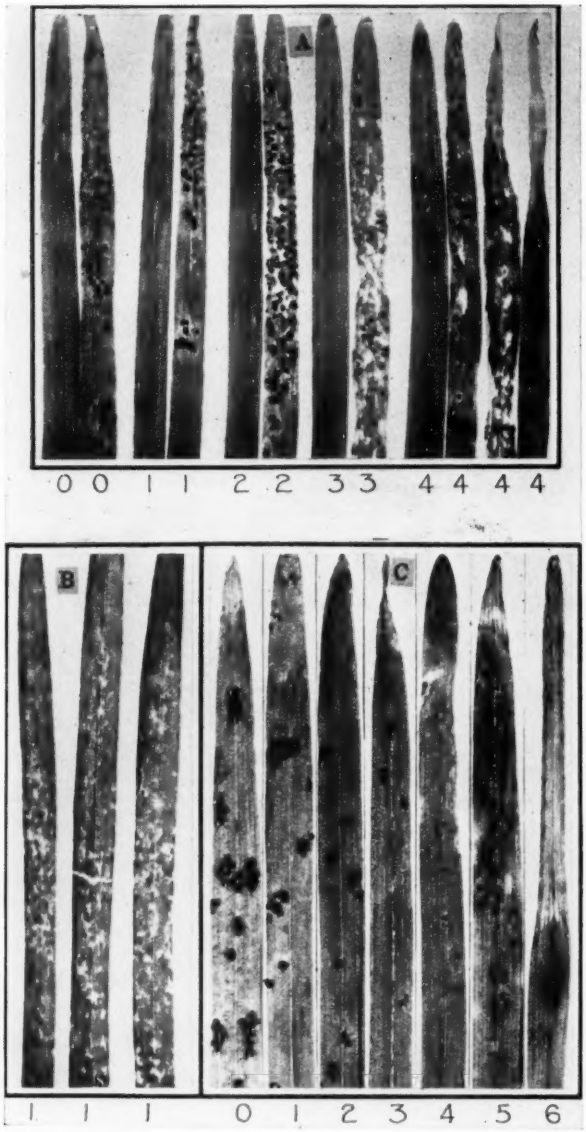


FIG. 8. A. Kota, Exp. 19, inoculated May 16, 1929, placed in dark May 22, photographed June 1. Left to right, 1 leaf each noninoculated and inoculated: untreated, in dark 1 day, 2 days, 3 days, 2 leaves noninoculated and 2 leaves inoculated in dark 4 days. B. Kota, Exp. 29, inoculated July 20, 1929, placed in dark July 24, photographed July 27. All 3 leaves in dark 1 day. C. Kota, Exp. 39, inoculated Oct. 7, 1929, placed in dark Oct. 13, photographed Oct. 26. Left to right: 1 leaf untreated, 1 leaf each in dark 1 day, 2 days, 3 days, 4 days, 5 days, 6 days.

ties that show necrosis under ordinary conditions, *e.g.*, Khapli (Fig. 4, A). The final effect, if darkness was sufficiently prolonged (Fig. 4, B, 4-day leaf), was the total suppression of externally visible signs of the rust, infection centers being marked only by areas of the leaf that remained green after long periods of subjection to darkness.

The intermingling of infection centers that were accompanied by hypersensitive tissue with others that were not must be ascribed to heterogeneity either of the infecting mycelia or of individual host cells or groups of cells, perhaps of both. When urediniospores from either susceptible or resistant pustules experimentally produced on the same leaf were transferred to new seedlings, the controls developed susceptible infection types, but when seedlings so infected were subjected to continuous darkness resistant and susceptible infection centers were again intermingled regardless of which type served as the source of inoculum. This suggests that the heterogeneity resides in the cell population of the wheat leaf rather than in the population of rust mycelia. In any one experiment increasing duration of darkness (within the limits defined in the preceding paragraph) increased the proportion of infections accompanied by hypersensitiveness. This, again, suggests that the cell heterogeneity of the host is of the nature of differences between individual cells or groups of cells in their rapidity of progression in the dark toward whatever physiological condition of the host tissue renders it incompatible with the fungus mycelium. As time in the dark increased, this condition was reached in more and more of the host cells, and the symptoms of incompatibility accompanied more and more of the infections. Heterogeneity amongst the cells of a single leaf in respect to the time relations of changes resulting from darkness is a phenomenon already recognized in the cherry laurel (12).

The full significance of the results recorded in this section can be estimated only in conjunction with certain experimental facts to be presented below, of which the chief is the fact that in the different experiments the appearance of resistant infection centers was not dependent upon the absolute duration of darkness but was correlated with the time in the dark required under any particular set of circumstances to cause the progression of the host tissue itself through its series of visible changes to the point of death. Before considering the experimental evidence upon which such a correlation is based, however, it is necessary to digress for the purpose of describing the effects of darkness on the number of pustules and flecks formed and to pay some attention to the part played in producing the results described in the present section by the period of time during which the plants were under natural conditions between removal from the dark chamber and the reading of the results recorded.

E. *Effect of continuous darkness upon the number of pustules and of total visible infection centers.* The imposed experimental treatment some-

times caused a reduction in the number of uredinia or sometimes even a reduction in the number of total visible infection centers, *i.e.*, pustules plus hypersensitive flecks. Since the rust fungus had equal chances of establishing infection in all plants before they were placed in the dark chamber, this can only mean that certain nests of mycelium were prevented from developing uredinia. Some of these caused the appearance of flecks on the leaves, while some produced no externally visible signs of their presence in the tissues.

The numerical reduction of pustules and flecks was especially evident among the resistant varieties. Actual counts were made for a few experiments when it was found that the number of pustules, the ratio of pustules to flecks, and the total number of visible infection centers diminished with increase in duration of continuous darkness.

It has already been stated, in describing experiments on resistant varieties, *e.g.*, number 72 (p. 515), that, in addition to its numerical effect on the infection centers, prolonged darkness caused a diminution in the size and the definition of hypersensitive flecks, resulting in a gradual progression toward invisibility with increase of time in the dark. It is not surprising, therefore, to find that some infections were so reduced as to be quite invisible from the exterior. This indicates that the effect of prolonged darkness upon resistant varieties constitutes a modification toward a state comparable to that designated by Stakman and Levine by the symbol "0", *i.e.*, a modification toward a more resistant type of infection than is represented in their usual "1" reaction.

The numerical relations, where susceptible host varieties were concerned, are not so clear. In several experiments, although a diminution in the ratio of pustules to flecks resulted from subjection to darkness, no significant decrease in the absolute number of pustules or of total visible infection centers occurred. In one experiment, on the other hand, reduction in the number of pustules and the total number of visible infection centers was definitely recognizable. In this latter experiment infection was very heavy, whereas in the former experiments it was light. It is not, however, possible to decide without many more observations than I have yet made if intensity of infection determines whether reduction in the number of visible infections occurs, nor, indeed, just what is the true effect upon numerical relations of the infections when hosts susceptible to the rust form employed are subjected to darkness.

F. *Secondary development of the rust.* A few days after the return of experimental plants to light following a period of darkness, pustules of a susceptible type began to appear in the tissue immediately surrounding many of the modified primary infections on susceptible hosts. These I shall refer to as secondary pustules, in contradistinction to those in the area of

primary infection by the fungus. The distinction was easily made when the primary infection involved necrosis, for the margin of the primary area was then sharply defined, and the primary pustule, when one appeared, was separated by necrotic tissue from the secondary pustule. There was, therefore, no difficulty in distinguishing the two until a fairly late stage of development, when the secondary pustules sometimes completely obscured the necrotic tissue. If, however, no necrosis appeared, a difficulty arose much earlier, for it was then not so easy to determine the limits of the primary-infection area. Sometimes, as in figure 3, e, the primary and secondary pustules remained distinct for a long time, but in other instances it was impossible to tell at the time when reactions would ordinarily be read which susceptible pustules were of primary origin and which partially or wholly of secondary origin. By making daily observations on marked leaves, however, it was possible to trace the history of their pustules and so to follow the course of secondary development.

The beginnings of secondary pustules did not on any occasion become externally visible less than 3 days after the return of the host plants to light, while the usual time was about a week. In certain instances it was nearer to 2 weeks. The development of secondary pustules in 2 Arnautka experiments is illustrated in figures 3, a–e. Figure 3, a, shows a leaf held in the dark 2 days (Experiment 34) with a necrotic fleck around which secondary growth of the rust was beginning when the leaf was photographed, 15 days after inoculation and 7 days after its return to light. Four days later this infection had attained the stage shown in figure 3, b. By this time the secondary growth was almost sufficient to hide the original fleck. Unfortunately, this particular infection was lost sight of, but observation of similar cases showed that the secondary growth might eventually completely hide the original fleck, giving the appearance to one who had not followed its history, of a completely susceptible type of pustule. The appearance of secondary pustules of a susceptible nature around necrotic flecks makes doubtful the theory that an area of necrotic tissue about an infection nucleus provides an impassable barrier to the rust mycelium and thus prevents its spread. It is evident that in the case under discussion the mycelium was able to reach the green tissue beyond the fleck, possibly before the death of the tissue involved in the fleck, and that it was there able to develop normally when conditions in the outer tissues were favorable. Figure 3, c, shows a leaf from experiment 64, in which secondary pustules had already hidden most of the primary pustules but had left the nature of the lowest one, a "3" pustule with a secondary ring, still evident. This illustration was made from a photograph taken 9 days after the 1-day period of darkness had been completed. Eleven days later the infection on the same leaf had reached the stage shown in figure 3, d. The lowest pus-

tule is here indistinguishable from the pustules of a "4" infection. Figure 3, e (Experiment 34), shows minute pustules with which no hypersensitive tissue was associated, being surrounded by secondary pustules bearing spores in abundance.

Figure 3, f, illustrates secondary growth on a Little Club leaf from experiment 66, held in the dark for 6 days and photographed 7 days after return to light. The primary infection was represented chiefly by rather poorly defined "nonnecrotic" flecks with faint green rings. Such a fleck without secondary pustules may be seen at the very base of the leaf (Fig. 3, f). The other infection centers on this leaf are represented by similar ill-defined flecks or by somewhat chlorotic areas surrounded by annular secondary pustules. These pustules later increased in size and finally completely obscured the areas of primary infection, producing an apparently susceptible infection type.

Secondary growth of the rust was partly responsible for the progressive change in the appearance of experimental plants, which has been referred to in recording the results of experiments and which made repeated observation of the plants necessary in order to be assured of recognizing the differences in infection type that could be attributed to different durations of continuous darkness in any experiment. In describing or photographing a leaf at any one time only, it is impossible to separate the effects of primary and secondary growth, and any momentary record of appearance must represent the resultant of the influence of the period of darkness acting together with the subsequent period of natural light conditions. It is in this light that photographs must be interpreted.

The rust infections on a single leaf did not always behave alike in the matter of developing secondary pustules. Sometimes resistant primary infection centers without secondary pustules appeared intermingled with those completely obscured by large pustules of secondary origin. The resulting appearance was suggestive of the standard "x" reaction, even though the primary infections had all been of a resistant type. The appearance of an "x" infection type might thus arise either from a mixture of primary pustules with and without accompanying hypersensitive tissue or from a set of uniformly resistant primary infections, some of which became obscured by secondary pustules of a susceptible type. After the "x" appearance had developed, it was not possible to tell in which way it had arisen unless one had followed the history of the individual pustules concerned.

The heterogeneity amongst primary infections in the matter of showing hypersensitive tissue or not has already been discussed, and evidence has been presented indicating that the cause of this heterogeneity probably lies within the host. Heterogeneity in the matter of sustaining secondary

growth, might also be a result of physiological heterogeneity amongst groups of host cells, of such a kind that the tissue around certain infection centers was capable of supporting secondary growth of the rust, following the treatment in the dark, while that around other infections was not. The alternative possibility must not, however, be ignored, namely, that some of the fungus mycelia within a leaf may have been killed by the effects of darkening, while others escaped destruction. This might again be referable to heterogeneity amongst host cells if the lethal effect came through the host, as is to be expected, and not directly through darkening the fungus. The question of heterogeneity with respect to secondary as well as to primary infection following subjection to darkness is one of very great interest but one which requires further experimental investigation before it can be understood.

*Recapitulation.* On the tissue of susceptible hosts that did not reach an irreversible state as a result of its experience in the dark, the rust developed secondary pustules of the type proper to its susceptible hosts under natural conditions in the greenhouse. This secondary development was not a change from resistant to susceptible on the part of the primary infection center itself but was an obscuring of the primary infections through envelopment by new growth. Sometimes not all of the infection centers on a leaf developed secondarily, with the result that a heterogeneous infection type was developed from a primarily resistant one.

The phenomena of secondary growth of the rust indicate that the alterations of host physiology that accompanied darkening and led to the modification of the primary-infection type were reversed upon return to light after a period of darkness that was not too prolonged, with the result that conditions approximating the original ones were ultimately reestablished except in those cells involved in the area of primary infection, where the presence of the fungus had caused an irreversible derangement or possibly the death of the protoplasm. As a result of this reversion, any subsequent growth of the rust led to a secondary infection type characteristic of the host under natural conditions. Thus it would appear that there is an association of congeniality or uncongeniality toward this form of rust with different physiological states of ordinarily susceptible host tissues, in both of which states these tissues are capable of existing under appropriate external conditions.

*G. Environmental influence upon changes in the appearance of host tissues and upon changes in the infection type.* Differences in response to experimental treatment greater than the individual differences amongst the leaves involved in a single experiment were encountered when a wheat variety was used at more than one time of year. In so far as these differences exceeded those in a single experiment, they must be attributed to variations in experi-

mental conditions. These were chiefly variations in environment during the growth of the plants, during confinement in the dark, and during the period following sojourn in the dark. The environment had its effect upon both type and rate of response. The age of the seedlings also affected the response to prolonged periods of darkness, and this factor is taken into consideration. The behavior of 3 varieties of wheat is described and illustrated.

KOTA—Kota was outstanding for the readiness with which it apparently responded to seasonal changes. It was used 8 times in the ordinary type of continuous-darkness experiment. Figures 7, B and C, 8, A, B, and C, and 11, C, show the response on most of these occasions.

1. EXPERIMENT 12b (not illustrated)—9 pots planted Apr. 10, 1929, inoculated April 20. To dark chamber April 25.

   EXPERIMENT 19 (Fig. 8, A)—20 pots planted May 6, 1929, 10 pots inoculated May 16. To dark chamber May 22. Photographed June 1.

   EXPERIMENT 62 (Fig. 7, B)—28 pots planted Mar. 7, 1930, 14 inoculated Mar. 20 (2½–3½ in. high). To dark chamber Mar. 28. Photographed April 5.

   *Reaction of controls*—"3" to "4 –."

   *Effect on infection type of continuous darkness for: one to six days*—green islands with necrotic halos producing a network of necrosis. Small pustules soon developed in green islands. In any one experiment, longer duration of darkness produced more necrosis, fewer and smaller pustules.

   *N.B.*—Differences in the 3 experiments were quantitative only. That is, in the March experiment (No. 62) it required 4 or 5 days in the dark to produce any considerable amount of necrosis that was not overgrown by secondary pustules at the ordinary time for reading reactions (15 to 20 days after inoculation); in the April experiment (No. 12) it required only 3 days, and in the May experiment (No. 19), 2 days (Figs. 7, B, 8, A).

   *Effect on host tissue of continuous darkness for: one, two days—as controls.*[12] *Three, four days*—in experiment 62 slightly pale; in experiments 12 and 19, pale, recovered only partially, some portions of leaves died. *Five, six days*—(in Experiment 62 only)—very pale, either recovered very slowly or died.

2. EXPERIMENT 29 (Fig. 8, B)—40 pots planted July 13, 1929, 20 pots inoculated July 20 (3 in. high). To dark chamber July 24. Photographed July 27, 7 days after inoculation, 2 days after return of the 1-day leaves to light.

   *Reaction of controls*—"3" (a few leaves "2++").

   *Effect on infection type of continuous darkness for: one day*—on return to light, network of necrosis about green islands as in figure 8, B. Later "2" reaction. *Two days*—smaller green islands, more necrosis. Fairly numerous pustules later. *Three days*—necrosis, only 2 small pustules on 18 leaves. *Four, five, six days*—no pustules, only small, green areas on yellow background. Leaves died without further development of rust.

   *Effect on tissue of inoculated plants of continuous darkness for: one day*—several leaves partly killed. *Two days*—most leaf tips, few whole leaves, killed. *Three*

[12] In all experiments, unless otherwise stated, the control plants were healthy in appearance.

*days*—more tissue killed, most of infected areas dead within a few days after removal from dark chamber. *Four, five, six days*—much tissue dead on removal from dark chamber, rest died very soon.

*Effect on noninoculated plants*[13] *of continuous darkness for: one, two days*—as controls. *Three days*—1 pot quickly recovered, 2 pots with much tissue killed. Parts of leaves not actually killed eventually recovered. *Four days*—only leaf bases greenish, much tissue killed, surviving parts recovered only partially. *Five, six days*—most of leaves were dead by 2nd day after return to light.

*N.B.*—One day in the dark was sufficient to cause the appearance of an extensive green-island development in this midsummer experiment, and longer periods of darkness were correspondingly more severe in their effect on rust development than equivalent periods in any of the spring experiments described.

3. EXPERIMENT 94 (Fig. 11, C)—36 pots planted Aug. 30, 1930, 16 pots inoculated Sept. 5 (2¼–4 in. high). To dark chamber Sept. 11, 9 pots subjected to continuous darkness, 3 control pots exposed to daylight. Photographed Sept. 21.

Detailed description on p. 543. In this late-summer experiment, carried out at a time when temperatures were high, short periods of darkness caused extensive necrosis, although the effect of equivalent periods of darkness was a little less severe than in experiment 29.

4. EXPERIMENT 39 (Fig. 8, C)—21 pots planted Sept. 28, 1929, 14 inoculated Oct. 7 (3½–4 in. high). To dark chamber Oct. 13. Photographed Oct. 26.

*Reaction of controls*—"3 ++".

*Effect on infection type of continuous darkness for: one, two days*—pustules slightly smaller. *Three days*—small pustules, often in indefinite nonnecrotic flecks. *Four days*—pustules smaller, trace of necrosis as in figure 8, C. *Five, six days*—minute pustules in nonnecrotic flecks, or green areas. Leaves pale, much tissue killed.

*N.B.*—No green islands with necrotic halos developed. With exception of trace referred to above, inoculated leaves showed no necrosis except such as occurred on noninoculated controls.

*Effect on host tissue of continuous darkness for: one, two days*—as controls. *Three days*—slightly pale. *Four days*—slightly pale, a few tips dead, one leaf dead. *Five days*—pale, several tips dead, several leaves ½ dead. *Six days*—very pale, about ½ of tissue dead.

5. EXPERIMENT 53 (not illustrated)—32 pots planted Dec. 3, 1929, 16 inoculated Dec. 16. To dark chamber Dec. 22.

Very light infection. Pustules on leaves deprived of light were small, not accompanied by necrosis ("3 ="). Six days or more in dark killed both infected and uninfected leaves.

6. EXPERIMENT 79 (Fig. 7, C)—14 pots planted May 16, 1930, inoculated May 24 (3–4 in. high). To dark chamber May 30. Photographed June 12.

*Reaction of controls*—"4 –" in appearance (from secondary growth about primary "3" pustules). Speckling on control and 1-day leaves distinct from flecking resulting from rust infection.

[13] For the most part the effect of darkness was essentially the same on noninoculated controls as on infected leaves; one description, therefore, serves for both. Only in such cases as the present one, where the two were essentially different, are separate descriptions included. The descriptions are in most cases from noninoculated plants.

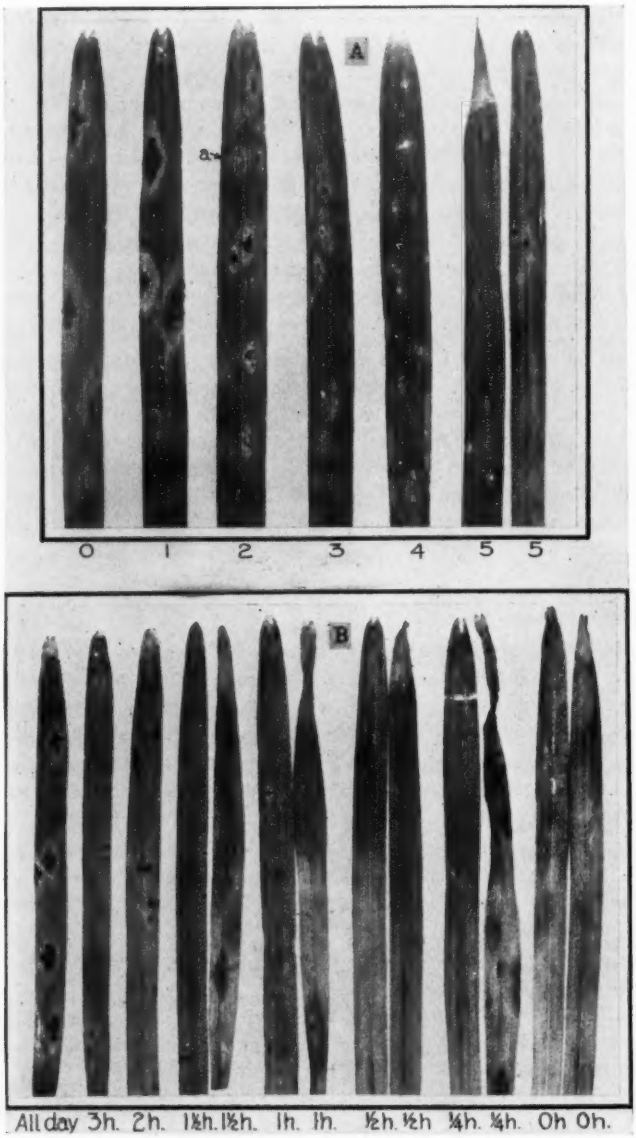


FIG. 9. A. Arnautka, Exp. 37, inoculated Sept. 29, 1929, placed in dark Oct. 6, photographed Oct. 16. Left to right, 1 leaf each: untreated, in dark 1 day, 2 days, 3 days, 4 days, 2 leaves in dark 5 days. a. Minute necrotic fleck with secondary ring forming. B. Arnautka, Exp. 36, inoculated Sept. 28, 1929, placed in dark Oct. 5, photographed Oct. 15. Left to right: 1 leaf exposed to full daylight, 1 leaf exposed daily for 3 hours, 1 leaf for 2 hours, 2 leaves for 1½ hours, 2 leaves for 1 hour, 2 leaves for ½ hour, 2 leaves for ¼ hour, over a period of 10 days, 2 leaves continuously in the dark for 10 days.

*Effect on infection type and host tissue of continuous darkness for: one day*—"4−" in appearance (from secondary growth about primary "3" pustules). A few leaves partly killed. *Two days*—few necrotic spots, not forming network. Slightly pale, tips pale, several leaves partly killed. *Three days*—small pustules in nonnecrotic flecks, chlorotic areas around pustules and flecks. Upper part of leaves pale, most with some dead tissue. *Four, five, six days*—leaves died almost immediately after return to light.

*N. B.*—Two days in the dark sufficed to cause appearance of a little necrosis accompanying rust infections; but, unexpectedly, the amount was not increased on the 3-day plants, on which nonnecrotic flecks appeared. (It is doubtful whether the large necrotic areas, such as appeared on one of the 1-day leaves in figure 7, C, can be attributed to the action of rust). It may be observed in connection with the behavior of these plants that light conditions in the greenhouse in the early part of their growth were not of the best. At the time of inoculation, a record was made to the effect that the growth was exceptionally tall and soft.

ARNAUTKA—Arnautka was used 7 times for continuous-darkness experiments. Figures 2, 3, 7, A, and 9, A, show the response on some of these occasions.

1. EXPERIMENT 17b (Fig. 7, A)—12 pots planted April 30, 1929, inoculated May 11 (4 in. high or more). To dark chamber May 17. Photographed June 3.

EXPERIMENT 28 (not illustrated)—40 pots planted July 9, 1929, 20 pots inoculated July 16. To dark chamber July 21.

*Effect of continuous darkness on infection type:* In July, earlier appearance of extensive hypersensitiveness was evident. Loss of a day's exposure to light sufficed to produce a good deal of hypersensitive tissue, chiefly as halos about green islands. In both experiments, appearance of minute necrotic flecks soon after removal from dark was recorded. These were soon obscured by secondary growth of rust. In all except these 2 experiments, the minute necrotic flecks were the chief insignia of incompatibility; hypersensitive halos were not prevalent, although they occasionally occurred in small numbers (Fig. 2, C).

*Effect of continuous darkness on host tissue:* In both experiments, 3 days in the dark caused paling of the leaves; longer exposures increased the degree of paling. In experiment 28, 2 days in the dark sufficed to kill a good deal of host tissue, while 3 days were required in experiment 17.

*N.B.*—In one respect figure 7, A, is misleading, *viz.*, in the effect of darkness upon the leaf tissue. Although the two 5-day leaves appear but slightly affected by the long dark period, these were the only 2 survivors out of 33 leaves. Some of the 3-day and 4-day leaves also were killed. It should be mentioned that, in selecting leaves for photographs, primary consideration was given to illustrating the effect of any treatment on rust-infection type. The effect upon general appearance of the leaves received secondary consideration.

2. EXPERIMENT 31 (not illustrated)—40 pots planted July 27, 1929, 20 pots inoculated Aug. 2 (2¾–3¾ in. high). To dark chamber August 9.

EXPERIMENT 34 (Fig. 3, a, b, c)—40 pots planted Sept. 6, 1929, 20 pots inoculated Sept. 12 (2–3 in. high). To dark chamber Sept. 18. Photographed Sept. 27, Oct. 1.

EXPERIMENT 37 (Fig. 9, A)—24 pots planted Sept. 20, 1929, 12 pots inoculated Sept. 29 (2½–3½ in. high). To dark chamber Oct. 6. Photographed Oct. 16.

EXPERIMENT 58 (not illustrated)—16 pots planted Jan. 2, 1930, inoculated Jan. 13 (3–5 in. high). To dark chamber Jan. 20.

EXPERIMENT 64 (Fig. 3, c, d)—49 pots planted Mar. 23, 1930, 28 pots inoculated Apr. 4 (2¼–3¼ in. high). To dark chamber Apr. 11. Photographed Apr. 21, May 2.

Figures 3, a, b, e, and 9, A are from Arnautka experiments of September, 1929, and represent as well the type of results obtained in January and April, 1930. In this type, loss of 1 day's exposure to light produced minute necrotic flecks, soon overgrown by secondary pustules to produce an almost or quite susceptible type of infection. Longer periods of darkness retarded growth of secondary pustules and also caused appearance of green islands, often, as in figure 2, c, around a necrotic fleck.

*N.B.*—In May, 1929, 3 or 4 days in dark sufficed to kill a considerable proportion of leaf tissue; in July, 2 days were enough. In August, 3 days were required, while in September, 1929, and in January, 1930, 4 or 5 days were necessary. The April, 1930, experiment (No. 64) is exceptional in that 1 or 2 days in dark sufficed to kill several leaves in April, when a less severe effect would be expected. It may be mentioned that in this experiment even control plants grew poorly and rust infection was very light.

LITTLE CLUB—Little Club was employed in 14 continuous-darkness experiments 2 of which are illustrated in figures 6, A, and 11, A.

1. EXPERIMENT 14c (not illustrated)—9 pots planted April 13, 1929, inoculated April 23. To dark chamber May 1.

   Two days in dark chamber caused appearance of a few tiny pustules in nonnecrotic flecks among the susceptible pustules and rendered leaves slightly pale; 3 days increased the proportion of flecks and the degree of paling. Longer periods of darkness caused appearance of minute green islands in many leaves and further increased degree of paling.

2. EXPERIMENT 20 (Fig. 6, A)—20 pots planted May 14, 1929, 12 pots inoculated May 22 (2–3 in. high). To dark chamber May 28. Photographed June 10.

   This experiment was similar in outcome to 14c. The only significant difference lay in the more rapid response to treatment; that is, 2 days in the dark at this time was apparently equivalent in its effect to 3 days in the earlier experiment. (p. 517).

3. EXPERIMENT 15c (not illustrated)—9 pots planted April 22, 1929, inoculated May 3. To dark chamber May 10.

   This experiment, intermediate in time between the 2 foregoing, responded as they did to shorter periods of darkness by production of nonnecrotic flecks intermingled with large pustules but differed from them in that green islands did not develop after longer periods in dark. Rather, the proportion of flecks to susceptible pustules increased with increasing duration of dark period. The behavior of experiment 15 was much commoner than that of the other 2 described. Indeed, it characterized most of the remaining Little Club experiments. In this experiment 4 days in dark were required to cause any noticeable degree of paling.

4. EXPERIMENT 24 (not illustrated)—42 pots planted June 8, 1929, 14 inoculated June 17. To dark chamber June 22.

EXPERIMENT 27 (not illustrated)—40 pots planted July 6, 1929, 26 pots inoculated July 12. To dark chamber July 17.

EXPERIMENT 30 (Fig. 11, A)—30 pots planted July 16, 1929, 15 pots inoculated July 24 (2¾–3¼ in. high). To dark chamber July 28. Photographed August 10.

These summer experiments of 1929 gave results like those of experiment 15. Experiment 30 is illustrated in figure 11, A. The response in experiments 24 and 27 was not entirely confined to the appearance of nonnecrotic flecks amongst the pustules, for a comparatively small amount of the green-island type of hypersensitiveness also appeared. In experiment 30, nonnecrotic flecks (Fig. 11, A) were the only insignia of resistance.

5. EXPERIMENT 42 (not illustrated)—72 pots planted Oct. 10, 1929, 24 pots inoculated Oct. 20 (3¼–3¾ in. high). To dark chamber Oct. 26.

EXPERIMENT 43 (not illustrated)—12 pots planted Oct. 14, 1929, inoculated Oct. 23. To dark chamber Oct. 29.

The 2 experiments started at the end of October were like number 30 in responding to continuous darkness by the appearance of flecks. But it required 4 or 5 days, in the dark chamber in the autumn to produce the hypersensitive flecks, while it required only 2 in July. The time required to kill any very great proportion of leaf tissue was also longer in the fall. The flecks produced in the autumn were perhaps more sharply defined than those produced in July, and some of them ultimately became necrotic.

6. EXPERIMENT 50 (not illustrated)—42 pots planted Nov. 20, 1929, 14 pots inoculated Nov. 29 (1¾–2¼ in.), 14 pots Dec. 1 (2½–3 in.), 14 pots Dec. 3 (3½–4 in.). To dark chamber Dec. 8, 10, 13, respectively. Under artificial light after removal from dark chamber.

EXPERIMENT 56 (not illustrated)—14 pots planted Dec. 18, 1929, 14 pots Dec. 21, 14 pots Dec. 24, 42 pots inoculated Jan. 2, 1930 (3¼–4¼ in.), (3–3¾ in.), (2¼–3 in.), respectively. To dark chamber Jan. 9. Under artificial light.

These 2 experiments conducted in December and January under artificial light each consisted of 3 lots of plants differing in age by 2 days in experiment 50 and 3 days in experiment 56. The type of rust response was much like that in October, although the flecks were not necrotic and were, for the most part, less well defined. The dark periods required to produce a visible response in rust infection were long, and host tissue was not visibly affected except by long sojourn in the dark. (p. 538).

7. EXPERIMENT 63 (not illustrated)—28 pots planted Mar. 11, 1930, 14 pots inoculated Mar. 29 (2–2¼ in. high). To dark chamber April 4.

EXPERIMENT 66 (Fig. 3, f)—28 pots planted Mar. 31, 1930, 14 pots inoculated Apr. 10 (2–2¾ in. high). To dark chamber April 16. Photographed April 29.

The early spring experiments of 1930 did not differ greatly from those conducted during the winter under artificial light. Rust response to experimental treatment consisted in the production, after several days in the dark, of nonnecrotic flecks surrounded by secondary circles of sporulating pustules (Fig. 3, f). In experiment 66 one of the 6-day leaves showed no secondary growth of rust but produced, rather, a small pustule in a necrotic fleck (a good "1" type). Five days of continuous darkness were required in both experiments to kill any considerable proportion of host tissue.

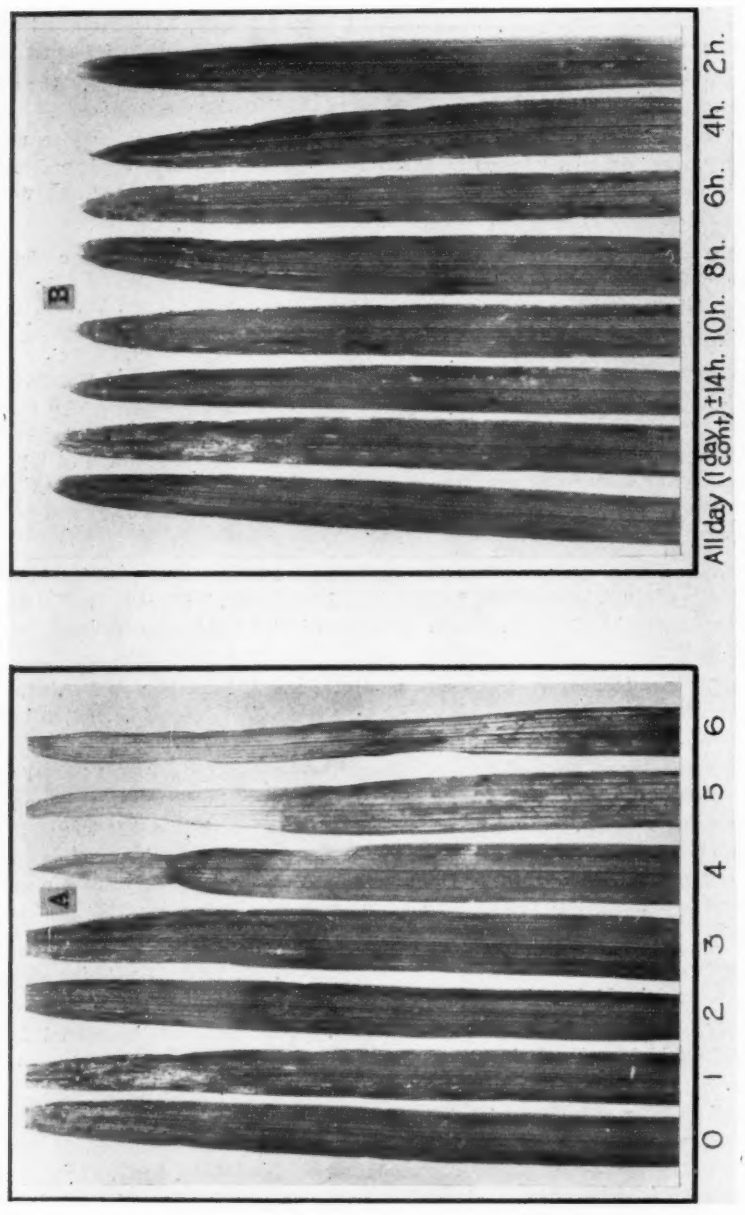


FIG. 10. A. Little Club, Exp. 30, uninoculated, placed in dark July 28, photographed Aug. 10. Left to right, 1 leaf each: untreated, in dark 1 day, 2 days, 3 days, 4 days, 5 days, 6 days. B. Little Club, Exp. 30, uninoculated, placed in dark July 28, photographed Aug. 10. Left to right, 1 leaf each, exposed to daylight: all day, about 14 hours, 10 hours, 8 hours, 6 hours, 4 hours, 2 hours, over a period of 13 days.

8. EXPERIMENT 74 (not illustrated)—13 pots planted April 8, 1930, inoculated April 19 (2¼–3 in. high). To dark chamber April 27.

In experiment 74 the flecks were also of 2 kinds, (1) nonnecrotic and rather indefinite or (2) conspicuous and necrotic. This experiment showed again the more rapid effect on appearance of leaf tissue that characterized the spring and summer experiments of 1929.

9. EXPERIMENT 71 (not illustrated)—42 pots planted April 12, 1930, 14 pots lot (b) inoculated April 24 (2½–3¼ in.), 14 pots lot (c) April 26 (3–3¾ in.). To dark chamber April 30, May 2, respectively.

The slightly older plants (c) reached the point of irreversibility (p. 502) faster than those 2 days younger and, correspondingly, required a shorter time in the dark to cause the production of hypersensitive flecks. Here the flecks were nearly all necrotic, large and well defined (Fig. 2, f). In 71b secondary growth made more progress than it did in 71c, but necrotic flecks were present here also. The severity of the effect of continuous darkness on rust infection increased in this experiment beyond the degree it had reached since the summer of 1929. This indicates a return to the conditions of the experiments of the previous late spring and summer and agrees with the behavior of the other varieties in showing, on the whole, more rapid change in the general appearance of host tissues and greater modifications of rust-infection type resulting from subjection to darkness in summer than from equally long subjection to darkness in winter. (p. 537).

*Recapitulation.* a. *Effect upon type of infection.* Each variety that was used several times for experimentation responded by the production of more than one infection type. In 4 out of 7 Kota experiments only green islands appeared, but in others green islands were, for the most part, replaced by nonnecrotic flecks (Figs. 7 and 8). Arnautka and Little Club produced minute green islands and extensive hypersensitive tissue under certain conditions (figs. 6, A, and 7, A). The minute flecks of the type shown in figure 9, A, were the only other symptoms of resistance displayed by Arnautka. Little Club also developed the Khapli type of infection (Fig. 2, f) and on many other occasions developed hypersensitive flecks, which did not resemble a standard Khapli infection so closely (p. 499; also Fig. 11, A). In certain experiments carried out in the fall or winter, *e.g.*, Kota, experiment 39 (Fig. 8, C) and experiment 53, no sharply hypersensitive tissue appeared after any period of darkness. This further emphasizes the effect of environment on the type of infection resulting from the subjection of seedlings to darkness. Environmental conditions during growth of the seedlings, the temperature prevailing in the dark chamber, and the conditions after the dark period ends must all be considered as possible factors influencing type of infection. It is probable that the environment during each of these three periods played its part in producing the protoplasmic states that determined the infection type on various occasions with the rust-host combinations involved.

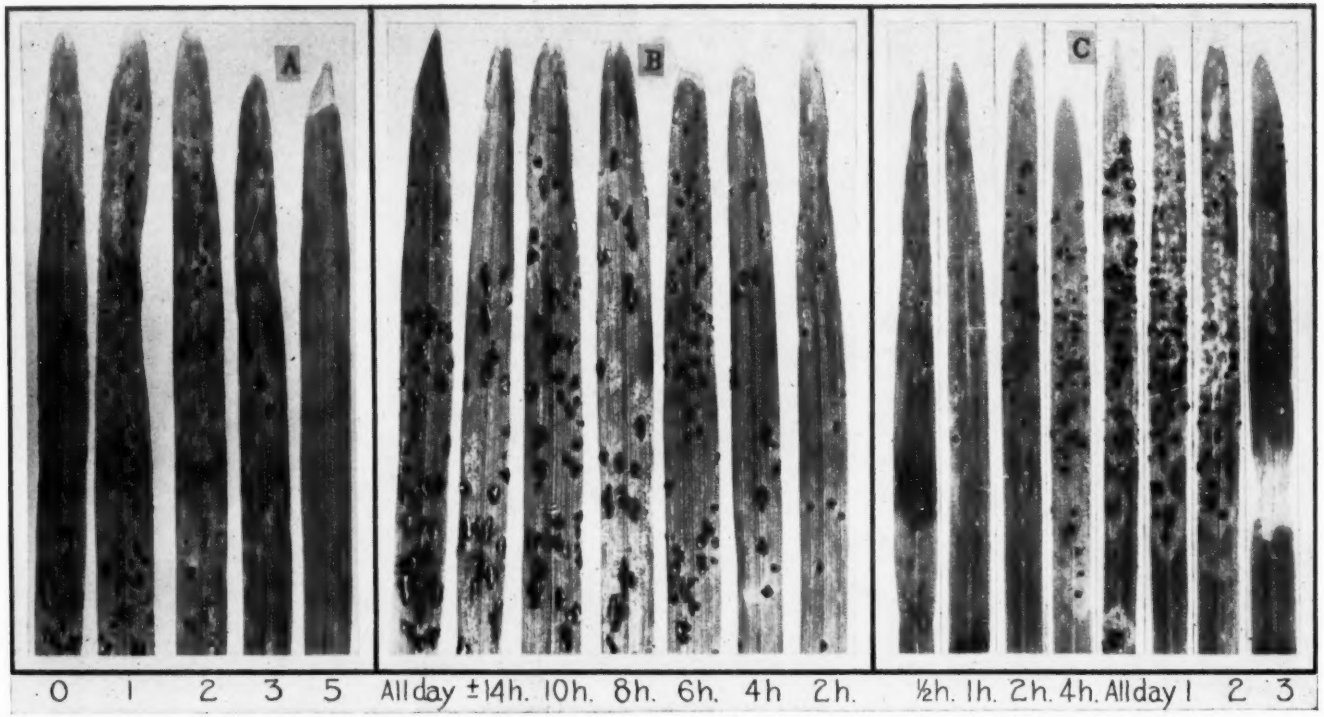


FIG. 11. A. Little Club, Exp. 30, inoculated July 24, 1929, placed in dark July 28, photographed Aug. 10. Left to right, 1 leaf each: untreated, in dark 1 day, 2 days, 3 days, 5 days. B. Little Club, Exp. 30, inoculated July 24, 1929, placed in dark July 28, photographed Aug. 10. Left to right: the same normal leaf as appears in figure 11, A, *i.e.*, exposed to daylight all day, 1 leaf each exposed to light daily for: about 14 hours, 10 hours, 8 hours, 6 hours, 4 hours, 2 hours, over a period of 13 days. C. Kota, Exp. 94, inoculated Sept. 5, 1930, placed in dark Sept. 11, photographed Sept. 21. Left to right, 1 leaf each: exposed to light daily for: ½ hour, 1 hour, 2 hours, 4 hours, all day; in continuous darkness for: 1 day, 2 days, 3 days.

There are certain evidences, also, of the importance of varietal or specific characters acting as internal factors in determining the type of infection under the experimental treatment here employed. In the first place, when, on a few occasions, two or three varieties were used for simultaneous experimentation, varietal differences showed up in seedlings of the same age and under identical environmental conditions. For example, in experiments 12a and b, inoculated April 20, 1929, the Little Club reactions following subjection to darkness were of the "x" type, while Kota produced its characteristic green islands. Kota's strong tendency toward green islands with a network of necrotic halos on seedlings subjected to darkness was, indeed, unique, although other varieties, including Little Club, developed green islands on certain occasions. Again, the particular type of necrotic fleck illustrated in figures 2, g; 2, h, and 9, A, might be called the durum type, for it occurred upon all durum varieties tested and was not encountered on any other variety. Furthermore, certain indications were found that Little Club required a longer period of darkness than any of the eight hosts under simultaneous test with it at one time or another, either to produce visible changes in host tissues or to bring about a change from compatibility to incompatibility with the rust fungus. Such indications are of interest because Little Club is more nearly completely susceptible to *Puccinia graminis tritici* as a whole than is any other of the differential host varieties. Further details concerning such evidence are omitted because of lack of space and because evidence of a more conclusive nature is being developed through experimentation in a controlled environment. Discussion of the matter is, therefore, reserved until the evidence is more complete.

b. *Effect upon time relations.* The rapidity of change in appearance of host tissues in the dark chamber and also the duration of darkness required to cause the subsequent appearance of a resistant infection type on any particular susceptible host variety differed under different sets of circumstances. The data concerning time relations in different experiments, as relating to the three varieties discussed in this section, are assembled in table 2. Column IV of this table presents the number of days' darkness required in each experiment to cause the subsequent death of any considerable proportion of host leaves, that is, to cause the death of more than the tips of a representative number of leaves in the pots receiving any particular treatment.[14] This time interval may be looked upon as a rough index to the rapidity of progression of dark metabolism to a point of irreversibility (p. 502). In many experiments values for noninoculated plants were obtained as well; but, since these are virtually the same as for inoculated plants, they are omitted. Column V represents the number of days in the

[14] Death subsequent to subjection to prolonged periods of darkness is progressive from tip to base (p. 502).

TABLE 2.—*A summary of experimental results showing correlation of the duration of darkness required to kill any considerable proportion of host tissue with the duration of darkness required to replace campatibility by incompatibility with Puccinia graminis tritici p.f.21*

| Variety | Exp. no. | Date of inoculation | No. of days of continuous darkness required to: | |
|---|---|---|---|---|
| | | | Kill host tissue[a] | Cause incompatibility[a] |
| I[e] | II | III | IV | V |
| Kota | 29 | July 20, 1929 | 1 | 1 |
| | 79 | May 24, 1930 | 2 | 2 |
| | 19 | May 16, 1929 | 3 | 2 |
| | 12b | Apr. 20, 1929 | 4 | 3 |
| | 62 | Mar. 20, 1930 | 5 | 4 or 5 |
| | 39 | Oct. 7, 1929 | 5 | —[b] |
| | 53 | Dec. 16, 1930 | 6 | —[b] |
| Arnautka | 28 | July 16, 1929 | 2 | 1 |
| | 31 | Aug. 2, 1929 | 3 | 2 |
| | 17b | May 11, 1929 | 3–4 | 2 |
| | 58 | Jan. 13, 1930 | 4 | 3 |
| | 37 | Sept. 29, 1929 | 5 | 3 |
| | 34 | Sept. 12, 1929 | 5 | 3–4 |
| | 64 | Apr. 4, 1930 | 1–2[d] | 3 |
| Little Club | 30 | July 24, 1929 | 3 | 2 |
| | 71c[c] | Apr. 26, 1930 | 3 | 4 |
| | 24 | June 17, 1929 | 3–4 | 2 |
| | 20 | May 22, 1929 | 4 | 2 |
| | 27 | July 12, 1929 | 4 | 2–3 |
| | 74 | Apr. 19, 1930 | 4 | 4 v. little |
| | 71b[c] | Apr. 24, 1930 | 4 | 5 |
| | 42w | Oct. 20, 1929 | 5 | 5 |
| | 56a[c] | Jan. 2, 1930 | 5 | 5 v. little |
| | 63 | Mar. 29, 1930 | 5 | 5–6 |
| | 66 | Apr. 10, 1930 | 5 | 6 |
| | 50b[c] | Dec. 1, 1929 | 5–6 | 5 v. little |
| | 42u | Oct. 20, 1929 | 6 | 5 |
| | 50a[c] | Nov. 29, 1929 | 6 or more | 5 |
| | 43 | Oct. 23, 1929 | more than 6 | 4 |
| | 56b[c] | Jan. 2, 1930 | 6–7 | 6 v. little |
| | 56c[c] | Jan. 2, 1930 | more than 7 | 6 v. " |
| Little Club | 50a[c] | Nov. 29, 1929 | 6 or more | 5 |
| | 50b. | Dec. 1, 1929 | 5–6 | 5 v. little |
| | 56c | Jan. 2, 1930 | more than 7 | 6 v. " |
| | 56b | Jan. 2, 1930 | 6–7 | 6 v. " |
| | 56a | Jan. 2, 1930 | 5 | 5 v. " |
| | 71b | Apr. 24, 1930 | 4 | 5 |
| | 71c | Apr. 26, 1930 | 3 | 4 |

[a] By "kill host tissue" is meant the killing of more than the tips of a representative number of leaves. By "cause incompatibility" is meant the production of hypersensitive tissue that remained visible 15–20 days after inoculation.

[b] No hypersensitive tissue appeared.

[c] In experiment 50a the plants were 9 days old when inoculated; 50b, 11 days old. In experiment 56a the plants were 15 days old; 56b, 12 days old; 56c, 9 days old. In experiment 71b the plants were 12 days old; 71c, 14 days old.

[d] See note on experiment 64, on page 530.

[e] The column numbers are used merely for convenience in referring to the table.

dark required in each experiment to cause a substantial failure of compatibility between the fungus and its host. Such a failure was regarded as having occurred when hypersensitive tissue (whether in the form of flecks or of halos about green islands) appeared in sufficient quantity to escape being totally obscured by secondary development of the rust 15 to 20 days after inoculation.

The time interval in column V is an index to the rapidity of change from compatible to incompatible relations between host and fungus, which provides a basis of comparison with the index to rapidity of metabolic change in the host presented in column IV. These indices make no claim to mathematical precision but are adequate for purposes of the present discussion. Rapidity of progression of dark metabolism to a state of irreversibility (resulting in the death of the tissue) and rapidity of change from compatibility to incompatibility are used in a sense reciprocal to the duration of darkness required to effect these changes. They bear no implications as to the manner in which they were brought about.

Considering the nature of the indices, the degree of correlation between the duration of darkness required to affect the host tissues irreversibly and that required to disturb the compatible relations between fungus and host seems highly significant. The numerical correlations are not exact, nor could they be expected to be. But when the experiments were arranged in such order that the values for each variety in column IV were in ascending order of magnitude, then, with only one or two exceptions, the values in column V were, also, in ascending order of magnitude. Where differences in column IV were small, a correlation with column V was not revealed; but, where differences in column IV were substantial, column V did not fail to show correlative differences.

It will be observed that, in general, the period of darkness requisite for the production of resistant infection types on these susceptible varieties was shorter in summer when both greenhouse and dark-chamber temperatures were high and change in the appearance of host tissue was rapid and was longer in winter when both greenhouse and dark-chamber temperatures were comparatively low and change in the appearance of host tissues was slower. An additional factor was found to be of some importance in affecting rapidity of change in general appearance of host tissues and in infection type. This was the age of the experimental plants. The effect of this factor is shown at the bottom of table 2 in the values given for experiments 50, 56, and 71. These three experiments will come under discussion later. To be remarked now is the strongly suggested correlation, whatever the factors determining it, between the duration of darkness required for the disturbance of compatibility between host and fungus and the duration of darkness required so to affect the host tissues as to cause death, although

the plants were returned to natural light conditions at the end of the dark period. The significance of such a correlation can only be that there is a common factor in the causation of the two effects. That this is a metabolic factor belonging to those processes in host metabolism that continue in the dark rather than to those that cease in the absence of light is a conclusion to which we are led by the following considerations: 1. More than a simple retardation of fungus growth resulted from the period of darkness; its relationship with the host tissue was so changed that all the signs of incompatibility appeared on hosts ordinarily entirely compatible with the fungus. 2. Appearance of symptoms of incompatibility on any susceptible variety did not depend upon the absolute duration of darkness. It was associated, rather, with the duration of darkness, widely different in different experiments, necessary to bring about an irreversible change in the appearance of the host tissues.

There is no implication that any definite appearance of a leaf is to be associated with loss of congeniality toward rust infection; indeed, the symptoms of incompatibility very often appeared in leaves not visibly affected in any way by their sojourn in the dark. It is intended here merely to imply that a darkened leaf, which passes rapidly through its series of visible changes, is passing rapidly through its course of metabolic changes, the early stages of which precede visible changes, and to point out that rapidity of this progression was associated in my experiments with rapidity of change from compatibility to incompatibility with the rust pathogen, whereas slow progression was associated with a slow change to incompatibility.

H. *Effect of slight differences in the age of the host upon changes in the appearance of host tissue and upon changes in the infection type.* Three experiments originally planned to detect, if possible, the causes of individual variation amongst leaves or amongst pots receiving the same experimental treatment substantiated the suggested correlation between metabolic change in darkened host tissues and disturbance of compatibility with the rust fungus employed. These three experiments dealt with plants differing but a few days in age when inoculated. Little Club wheat was used for all of them.

Two of the three experiments were carried out in the winter of 1929–30 on plants inoculated at the ages of 9, 11, 13 days for experiment 50 and of 9, 12, 15 days for experiment 56. The effects of continuous darkness for periods of from 1 to 7 days were much alike as to rust-infection type on plants of all the ages tested. Indications of a slightly more severe effect on the older plants, however, were found. The tissues of the older host plants showed more rapid progression through their characteristic series of visible changes in the dark than did those of the younger plants.

The third experiment, No. 71, was carried out in the spring of 1930. Plants were inoculated at the ages of 12 and 14 days. Necrotic flecks (Fig. 2, f) developed after a shorter period of darkness and more abundantly after equivalent periods of darkness on the older plants than on the younger (p. 533). At the same time there resulted from subjection to darkness a more rapid progression to death of the tissues of the older plants (Table 2). Removal of the endosperm at the time of inoculation from ½ the plants receiving each treatment revealed no differences resulting from this operation. This suggests that the endosperm was not responsible for whatever change with age in the physiological constitution of the seedlings accounted for the changes in response to darkness, for a difference of 2 days in the age of the seedlings was correlated in this experiment with more pronounced differences than were seen in either of the winter experiments, both as to rapidity of progression of the leaf tissues to death following subjection to darkness and as to rapidity of change in the dark from compatibility to incompatibility with the rust pathogen involved.

It seems important to know the nature of the rapid change in physiological constitution of the seedlings that must underlie these differences. In the first place, the differences that appeared to depend on slight inequality in the age of the seedlings were of the same sort as occurred amongst individual plants throughout the experiments, and it is possible that variation in the rates of physiological aging of the seedlings used for a single experiment can be shown to account for at least a part of the individual variations observed.[15] In the second place, this rapid change in physiological constitution of the seedlings affords further instances of a correlation of the duration of darkness required to cause death of host tissue with the duration required to disturb compatible relations with the rust form involved and introduces age of tissue as a possible factor in determining both of these quantities. This relation of age of host to effect of darkness on host tissue and on rust infection suggests that experimentation with plants of wider age differences might provide valuable information concerning the possible existence and nature of a physiological element in mature resistance. It may be pointed out that the age of the tissue or organ concerned is known to affect the time relations and even the course of dark metabolism in other plant tissues (12), (2), and (3).

### II. EXPERIMENTS ON SEEDLINGS WITH SHORT DAILY EXPOSURES TO LIGHT

A. *Conditions of experimentation.* Plants were inoculated as usual and the rust was allowed to establish itself before experimentation began.

[15] Preliminary experiments in which plants were grown in water culture instead of in soil and one experiment for which Little Club seeds were selected within a narrow range of weight failed to reduce individual variation or to reveal any indications of its cause.

When infection flecks first appeared, the experimental pots were transferred to the dark chamber whence they were transferred to the greenhouse bench for the desired number of hours each day. This was continued either throughout the period of development of the rust or, for the sake of comparison with this treatment, for a few days only, after which the plants were left continuously on the greenhouse bench. The exposures to light usually were arranged so that they centered around noon. For instance, a 10-hour exposure began at 7:00 a. m. and ended at 5:00 p. m. The longest and shortest exposures were sometimes exceptions to this. For instance, one exposure was from 7:00 a. m. to dark, or about 9:00 p. m.; ¼-hour exposures were usually from 11:15 to 11:30 a. m.; and ½-hour exposures from 11:00 to 11:30 a. m.

B. *Effect of short daily exposures to light upon the appearance of the host tissues.* Figure 10, A and B, compares the effects of continuous darkness and of short daily exposures on Little Club seedlings inoculated in July, 1929. A detailed description of the effect of the two experimental treatments in this particular case is to be found on page 542. It is obvious (Fig. 10, B) that interruption of darkness by even a short light period once in 24 hours prevented the progress of dark metabolism to the point where any visible change in the leaves appeared. Even 2 hours of light a day over a period of 13 days sufficed to prevent the slightest paling of the leaves in 3 out of 4 pots so treated. Reference to the descriptions of other experiments of the same nature involving other wheat varieties as well as Little Club (pp. 542–545) will show that 4-hour light periods daily were in nearly all cases sufficient to prevent any visible change in the host tissues. Usually shorter periods of exposure eventually caused progressive paling, loss of turgor, and finally death of the tissues. The same gradient in rapidity of change from tip to base of the leaf was found here as was recorded for plants held continuously in the dark. Even short exposures to light, however, delayed these visible changes in the leaves considerably beyond the time when they appeared on corresponding leaves held continuously in the dark.

C. *Effect of short daily exposures to light upon rate of rust development.* Table 3 has been prepared in the same way as was table 1. In it are collected the data on rate of development of pustules for all experiments on short daily exposures to light. As before, column IV represents the number of days required for the first appearance of infection flecks after inoculation, and the remaining columns the number of days from the appearance of infection flecks until the majority of pustules were formed as swellings under the epidermis. The most striking information contained in the table is that the rust developed just as fast with a very few hours' exposure to daylight each day as it did when the plants were exposed all day. Kota,

TABLE 3.—*A summary of experimental results concerning the effect of short daily exposures to light upon the rate of pustule development of Puccinia graminis tritici p.f.21 on 5 differential host varieties*

| Variety | Exp. No. | Date of inoculation | Time from inoculation to appearance of flecks | Lapse of time in days between the first appearance of infection flecks and the stage when most of these had formed pustules, with daily exposures to light of the following durations: | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | All day | 8:00 a. m. dark | 9:00 a. m. dark | 10 hr. | 8 hr. | 6 hr. | 4 hr. | 3 hr. | 2 hr. | 1½ hr. | 1 hr. | ½ hr. | ¼ hr. | 0 hr. |
| I[c] | II | III | IV | V | VI | VII | VIII | IX | X | XI | XII | XIII | XIV | XV | XVI | XVII | XVIII |
| Little Club | 30 | July 24, 1929 | 4 | 3 | 3 | ...[a] | 3 | 3 | 3 | 3 | ... | 4–5 | ... | ... | ... | ... | –[b] |
| | 41 | Oct. 17, 1929 | 6 | 3 | ... | ... | ... | ... | ... | 4–5 | ... | 5 | 6–8 | 7–9 | ... | ... | – |
| Kota | 94 | Sept. 5, 1930 | 6 | 2 | ... | ... | ... | ... | ... | 2 | ... | 2 | ... | 2 | 2–3 | ... | ... |
| Arnautka | 36 | Sept. 28, 1929 | 7 | 3–4 | ... | ... | ... | ... | ... | ... | 4 | 3–4 | 3–4 | 3–4 | – | – | – |
| | 45 | Nov. 8, 1929 | 6 | ... | ... | ... | ... | ... | ... | 4–6 | ... | 4–5 | ... | 5 | – | – | – |
| Einkorn | 38 | Sept. 30, 1929 | 7 | 5 or more | ... | ... | ... | ... | ... | ... | ... | – | ... | – | – | ... | ... |
| Khapli | 35 | Sept. 14, 1929 | 8 | 4 | ... | 4 | ... | 4 | 4 | 4–5 | ... | – | ... | – | – | ... | ... |

[a] A series of dots (...) signifies that the data required to fill a certain space in the table are lacking.

[b] A minus sign (–) signifies that few or none of the infection flecks developed pustules as long as the plants were kept under observation or until they died.

[c] The column numbers are used merely for convenience in referring to the table.

inoculated in September (Experiment 94), showed no retardation of pustule development when the exposure was reduced to 1 hour per day, and retardation was doubtful even with ½ hour exposure. Little Club, inoculated in July (Experiment 30), showed the first retardation when the exposure was reduced to 2 hours per day, while in October (Experiment 41) plants exposed 4 hours a day required 1 or 2 days longer to develop pustules than those exposed all day. Arnautka (in Experiments 36 and 45, both in the late fall or winter) showed no retardation when the exposure was reduced to 1 hour a day. With shorter exposures pustule development was almost entirely suppressed. As with Arnautka, the effect of very short exposures to light on Einkorn and Khapli was to suppress pustule development; and, since only short exposures and full daylight were used with Einkorn, no information concerning rate of development is available. The Khapli experiment shows that daily exposures as short as 4 hours did not influence the rate of development of the rust on this variety.

D. *Effect of short daily exposures to light upon the infection type.* Experiments involving Little Club, Arnautka and Kota all failed in the production of well-defined hypersensitive tissue following short daily exposures of the experimental plants to light. This offers a sharp contrast to experiments described here for the sake of comparison, wherein continuous darkness, as in experiments recorded earlier, caused the appearance of sharply hypersensitive tissue on these host varieties. Resistant varieties responded to short daily exposures to light much as they did to continuous darkness, that is, by reduction in pustule size and number and in definition of flecks.

LITTLE CLUB, EXPERIMENT 30 (Figs. 10 and 11). Sixty pots planted July 16, 1929, 30 pots inoculated July 24 (2¾–3¼ in. high). Infection flecks appeared July 28 and all but control pots placed in dark chamber. Some were left in dark chamber continuously for periods of 1 to 7 days, then returned to the greenhouse; that is, they were subjected to continuous darkness. Others were removed from dark chamber daily for periods of 2, 4, 6, 8, 10, and about 14 hours and were kept in dark the rest of the time; this treatment was continued for 13 days. Photographed Aug. 10, the 13th day after initiation of treatment and 17th day after inoculation.

*Effect on infection type:*

*Continuous darkness* (Fig. 11, A):—Nonnecrotic flecks even after 1 day in dark; increased in proportion to number of pustules with increasing periods of darkness.

*Short daily exposures to light for 13 days* (Fig. 11, B): *Exposed: 14 ±, 10, 8, 6, 4 hours daily*—no effect on the appearance of the rust. *Two hours daily*—reduction in size of pustules. No change in infection type. No hypersensitiveness. (The ill-defined whitish areas on some leaves were not effect of rust infection. They were present on many noninoculated leaves and can be seen in figure 10. They happen to be fairly numerous on the leaf kept in the dark 1 day. These were readily distinguishable from the nonnecrotic flecks reported on leaves subjected to periods of continuous darkness).

*Effect on host tissue:*

*Continuous darkness* (Fig. 10, A): *1, 2 days*—no significant change in appearance. *Three days*—a few leaves were irreversibly affected (p. 502), most of them not seriously damaged. *Four days*—much tissue killed, surviving tissue quite pale. *Five, 6 days*—most of the leaves killed.

*Short daily exposures to light for 13 days* (Fig. 10, B): *Exposed: 14 ±, 10, 8, 6, 4 hours daily*—no effect on appearance. *Two hours daily*—in 1 pot, no effect, in 1 pot the leaves became pale and flaccid after 8 days of this treatment and finally died. (The 2 pots of inoculated plants exposed to light 2 hours daily behaved as did the first pot mentioned; that is, they showed no deleterious effect of prolonging their nightly periods of darkness to 22 hours, thus shortening their daily light periods to 2 hours).

LITTLE CLUB, EXPERIMENT 41 (not illustrated)—32 pots planted Oct. 8, 1929, 16 pots inoculated Oct. 17 (2½–3 in. high). Exposures 4, 2, 1½, 1, ½, ¼ hours per day—2 types of experimental procedure: (1) Short daily exposures continued 9 days and then plants returned to natural conditions. (2) Short daily exposures continued 19 days.

*Effect on infection type of both treatments:*

*Exposed to light: All day*—"4+". *Four hours daily*—no change. *Two hours daily*—on some leaves no change, except in reduction of size of primary rust pustules; on some leaves some effect on host tissue occurred that might be classed as chlorosis, it was of a diffuse type, and not sufficiently defined to be classed as hypersensitive tissue. *1½, 1, ½ hours daily*—pustules smaller, no definite flecks. *One-fourth hour daily*—on 1 leaf so treated for 9 days 1 rather definite non-necrotic fleck developed by the 26th day after the initiation of the treatment. (Throughout these experiments with short daily exposures to light, leaves exposed only ¼ hour daily did not behave very differently from those held continuously in the dark. Such a short exposure evidently did not allow for much physiological readjustment in leaf tissue. The same applies to behavior, on some occasions, of leaves exposed ½ hour daily.)

*N.B.*—Throughout the experiment, the difference between plants confined to short periods of light for 9 days and those so treated for 19 days lay entirely in the presence of secondary rust growth on the former and its absence on the latter 19 days after transfer of the plants to the dark. Seven days later, secondary growth was present on the 19-day-treated plants as well, although after exposures less than 2 hours a day such growth did not advance very far.

*Effect on host tissue:*

*Nine-day treatment: Exposed: 4 hours daily*—like plants exposed all day. *Two, 1½ hours daily*—became slightly pale. *One hour daily*—became pale, a good deal of tissue died. *One-half, ¼, 0 hours daily*—pale, died a few days after return to natural light conditions.

*19-day treatment: Exposed: 4 hours daily*—like plant exposed all day. *Two, 1½ hours daily*—became slightly pale. *One hour daily*—pale, several tips or ends died. *One-half, ¼, 0 hours daily*—died after about 14 days.

KOTA, EXPERIMENT 94 (Fig. 11, C)—36 pots planted Aug. 30, 1930, 16 pots inoculated Sept. 5, (2¼–4 in. high). To dark chamber Sept. 11. Nine pots subjected to continuous darkness, 4 pots held in dark chamber for 11 days with daily exposures to

light of 4, 2, 1, ½ hours per day. Three control pots exposed to natural light conditions. (All but the 16 pots inoculated were destroyed by a squirrel). Photographed Sept. 21.

*Reaction of controls:* "3+" to "3++".

*Effect on infection type:*

*Continuous darkness: 1 day*—2 out of 3 leaves with green islands and necrotic halos. *Two days*—more necrosis as halos about green islands. *Three days*—as 2 days, but fewer pustules and fewer green islands. *Four, 5 days*—a few green areas with minute flecks, died very soon after removal from dark chamber.

*Short daily exposures for 11 days: Exposed: 4 hours daily*—"3+". *Two hours daily*—"3". *One hour daily*—"3=". Some minute pustules in green areas; no necrosis. *One-half hour daily*—minute pustules in green areas, no necrosis.

*Effect on host tissue:*

*Continuous darkness: 1 day*—as controls. *Two days*—slightly pale. *Three days*—pale, a few tips dead. *Four, 5 days*—pale, upper parts almost white, soon dying.

*Short daily exposures for 11 days: Exposed: 4 hours daily*—as controls. *Two hours daily*—2 leaves as controls, 1 pale and flaccid. *One hour daily*—pale, infected areas green, 2 tips flaccid. *One-half hour daily*—completely etiolated, infected areas green, becoming flaccid.

ARNAUTKA, EXPERIMENT 36 (Fig. 9, B)—30 pots planted Sept. 19, 1929, 15 pots inoculated Sept. 28 (2½–3½ in. high). (Some of the plants were reinoculated the next day because they were flooded in the inoculation chamber, and the spores were possibly washed off the leaves). To dark chamber Oct. 5. Kept in the dark as long as they survived with daily exposures to light of 3, 2, 1½, 1, ½, ¼, 0 hours. Control pots exposed all day. Photographed Oct. 15, 10 days after initiation of treatment.

ARNAUTKA, EXPERIMENT 45 (not illustrated)—24 pots planted Oct. 29, 1929, inoculated Nov. 8. To dark chamber Nov. 14. One-half of experimental pots kept in dark chamber for 5 days, ½ for 14 days, with daily exposures to light of 4, 2, 1, ½, ¼, 0 hours. Control pots exposed all day.

*Effect on infection type:*

Arnautka provided a very striking example of the different effects of continuous darkness and short daily exposures to light. Figure 9, A illustrates experiment 37 (p. 529), a continuous-darkness experiment carried out almost simultaneously with experiment 36, the short-exposure experiment illustrated in Figure 9, B. (Inoculations were 1 day apart). The outstanding feature of the infection on leaves exposed to light for short periods daily is the total absence of necrotic flecks. A reduction in the size of the pustules resulted from this treatment, and in the 1-hour leaves a diffuse nonnecrotic effect upon the host tissue appeared (Fig. 9, B). On the other hand, many of the leaves subjected to continuous darkness in experiment 37 bore sharply necrotic flecks. Figure 9, A does not show such flecks on 1-day leaves, but they were present earlier on these leaves and were overgrown by secondary pustules before the photograph was taken. On 2-day leaves (at *a*) and on 3-day and 4-day and 1 of the 5-day leaves they can be seen. It will be observed from figure 9, B that leaves of experiment 36 exposed continuously to dark produced no necrotic flecks. These leaves were, however, 10 days in the dark before being photographed. In a previous section, it was pointed out that necrotic flecks do not appear after very long periods in the dark. Even Khapli

lost them in experiment 22 after 5 days in the dark (Fig. 4, A), and Figure 9, A, shows that, in experiment 37, even after 5 days of continuous darkness, Arnautka did not produce necrotic flecks on all of its leaves. The same thing was demonstrated by experiment 45, a 2nd short-exposure experiment with Arnautka, in which inoculations were made on Nov. 8, 1929. On this occasion, leaves kept continuously in the dark for 5 days produced necrotic flecks, while those kept there for 10 days produced none. The leaves were dead before 14 days were over, but a few survived for 10 days. Plants of the same experiment exposed to light for daily periods of ½, 1, 2, 4 hours, respectively produced no necrotic flecks, whether this treatment was continued for 5 or 14 days. In development of pustules they resembled experiment 36 very closely. Necrotic flecks were according to these observations, characteristic of Arnautka plants only after sufficient continuous sojourn in the dark. When darkness had been too prolonged (more than 5 days) or too short (less than 24–36 hours), the flecks did not appear.

*Effect on host tissue:*

Experiment 45:

*5-day treatment: Exposed to light: 4 hours daily*—no effect. *Two, 1 hour daily*—slightly pale, recovered. *One-half, ½ hour daily*—pale, recovered color partially, most tissue died. *Subjected to continuous darkness for 5 days*—pale, partially recovered, much tissue died.

*Fourteen-day treatment: Exposed to light: 4 hours daily*—very slightly pale. *Two hours daily*—slightly pale, ends dead. *One, ½ hour daily*—very pale, much dead tissue. *One-fourth hour daily*—nearly all dead. *Subjected to continuous darkness*—all dead after 14 days, a few survived 10 days.

Experiment 36:

*Exposed to light: Three, 1½ hours daily*—became pale after about 10 days. *One, ½ hour daily*—became very pale, much tissue died.

EINKORN, EXPERIMENT 38 (not illustrated)—36 pots planted Sept. 21, 1929, 17 pots inoculated Sept. 30 (3½–4 in. high). To dark chamber Oct. 7. Fourteen inoculated and 14 noninoculated pots subjected to continuous darkness, 3 inoculated, and 3 noninoculated pots kept in dark with daily exposures to light of 2, 1, ½ hours.

KHAPLI, EXPERIMENT 35 (not illustrated)—28 pots planted Sept. 6, 1929, 15 pots inoculated Sept. 14 (4–5 in. high). To dark chamber Sept. 22. One-half kept in dark 8 days, ½ 18 days, with daily exposures to light of about 14, 8, 6, 4, 2, 1, ½ hours.

*Effect on infection type and host tissue:*

Einkorn and Khapli are both highly resistant to form 21 and under conditions of short exposures to light responded much as they did to periods of continuous darkness, that is, by reduction in 3 respects, proportion of flecks that bore pustules to those that did not, size of flecks, and definition of flecks. Khapli plants exposed to light ½ hour daily were much like the 5-day plants of Figure 4, A. Plants exposed 1 hour daily produced almost no pustules. They presented an appearance similar to that of the 4-day plants in figure 4, A, while the 2-hour leaves were much like those kept 2 or 3 days continuously in the dark. Exposure to light for 8, 6, and 4 hours, respectively, produced an infection not observably different from plants allowed to develop under greenhouse conditions. In general, the same statement is true for Einkorn as for Khapli. As with the other varieties discussed, only in plants receiving the shortest light exposures did changes in the appearance of the host tissue occur.

*Recapitulation.* Under the conditions of these experiments, long periods of darkness when interrupted frequently by short periods of light did not produce the same effect as shorter periods of uninterrupted darkness. Four-hour exposures to light daily sufficed to prevent any marked differences in either rate or final appearance of rust development from those observed under natural conditions of illumination in the greenhouse. Shorter daily exposures to light caused retardation of development and reduction in pustule size but no distinctly hypersensitive tissue, although continuous subjection to darkness for 36 hours, or, in some instances longer, caused the appearance of well-defined hypersensitive flecks or halos on plants quite comparable to those receiving the short exposure treatment. Four-hour light periods daily were sufficient to prevent any effect of prolonged darkness on the external appearance of the leaves, and shorter light periods delayed the paling and subsequent changes resulting from darkening of the seedlings.

Except on the theory that a disadjustment of compatible relations between host and fungus is dependent upon the metabolic sequence in darkened tissue, it would be very surprising that plants continuously in the dark 2 nights and 1 day should develop even a slight amount of definitely hypersensitive tissue, while corresponding plants almost continuously in the dark 13 nights and days, with only a 2-hour exposure to daylight once in 24 hours, should develop none. The plants that were in the dark 2 nights and 1 day were subjected to a longer *continuous* period of darkness than any of those exposed to light once in 24 hours, although the *total* sojourn of the latter in the dark greatly exceeded that of the former. For instance, in experiment 30 with Little Club, on the basis of a 14-hour day and a 10-hour night, the 1-day plants had received a total of approximately 150 hours of darkness from the time of their first transfer to the dark chamber until they were photographed (Fig. 11, A), the 2-day plants had received approximately 175 hours, and the plants exposed to light 4 hours daily had received over 250 hours of darkness. Yet the 1-day and 2-day plants bore hypersensitive tissue, while those exposed to light 4 hours daily not only bore no hypersensitive tissue (Fig. 11, B) but supported a rust development indistinguishable in any respect from that of control plants under natural conditions of illumination. The longest *continuous* period of darkness to which these latter plants were subjected, however, was 20 hours, while the 1-day plants had one period of continuous darkness extending over 2 nights and 1 day, or approximately 36 hours, and the 2-day plants (bearing more hypersensitive tissue) had one period of approximately 60 hours in the dark.

In the face of these facts we can only conclude that the appearance of hypersensitive tissue was associated with some physiological condition of the host protoplasm not attained during recurrent 20-hour periods of darkness

but attained by a few cells after a period of 36 hours in the dark and by a much larger proportion of the leaf tissue after 60 hours in the dark. The absence of any correlation between the sum total of time in the dark and the appearance of hypersensitive tissue argues strongly against the possibility that cessation of carbon assimilation was alone responsible for the disturbance of compatible relations between host and fungus.

The cessation of carbon assimilation cannot, of course, be without its effect, for photosynthesis ordinarily provides a substrate for metabolic processes that are continued in the absence of light, and, in so far as low concentration of substrate for these reactions contributes to the altered course of metabolism, referred to here as dark metabolism, to that extent must the cessation of photosynthesis be held responsible for the changes in the relations of the host toward the rust. Dark metabolism, however, if allowed to proceed for a sufficiently long time, comes to involve physiological states that do not occur in the same host under otherwise similar conditions if it is permitted to assimilate periodically. The evidence from the experiments described above indicates that one (or possibly more) of these states, rather than the mere absence of the processes of carbon assimilation, is responsible for a change on the part of the host from congeniality to uncongeniality toward the rust form involved.

### III. EXPERIMENTS ON DETACHED LEAVES WITH CONTINUOUS PERIODS OF DARKNESS

A. *Conditions of experimentation.* Seedlings were grown and inoculated as usual and the rust was allowed to develop until infection flecks appeared on the leaves. Then, only when they were ready to be transferred to the dark chamber were infected and noninfected leaves cut off and floated on distilled water in Petri dishes or placed in vials with their bases dipping into water. They were then held in darkness continuously for various periods of time. Two such experiments were carried out simultaneously with experiments on seedlings.

B. *Effect of continuous darkness on the appearance of host tissues.* Details of the effect on host tissues accompany those on infection type in the following section. The course of visible changes was remarkably different in the leaves detached at the time of transfer to the dark from that in seedlings transferred in pots to the dark. In the first place, the gradient from more rapid change at the tips to slower change at the base, which so constantly appeared in attached leaves, was entirely absent from detached leaves. The tips were often the last portion of the detached leaves to lose their green color, and a greater degree of uniformity throughout the main portion of the leaf existed here than in attached leaves. Rates of change also were different, as can be seen from a comparison of the detailed descrip-

tions of detached leaves and seedlings receiving the same treatment. In experiments 47 and 48, for instance, attached leaves held for 6 days in the dark were only very slightly pale, while detached leaves were almost completely etiolated. While such very striking discrepancies did not appear in experiment 96, a comparison of the descriptions of detached and attached leaves on page 56 will reveal certain very distinct differences.

This sequence of outwardly visible changes in host tissue must be taken as indicating a different metabolic course from that obtaining when whole plants are held in the dark. This indication has already found corroboration in the results of certain experiments carried out by G. H. Duff and myself on the respiratory behavior of attached and detached leaves during long periods of dark, for they disclose some interesting differences between the metabolic courses of the attached and detached leaves.

C. *Effect of continuous darkness on the infection type.* Differences in the effect of darkness on infection type were found associated with the diverse metabolic courses just referred to. On detached leaves from plants subjected to darkness rust development was faster than on attached leaves and was not accompanied by the development of hypersensitive tissue.

LITTLE CLUB, EXPERIMENTS 47, 48 (not illustrated)—54 pots planted Nov. 6, 1929, 27 pots inoculated Nov. 15 (2–2½ in. high). To dark chamber Nov. 22.

*Detached leaves in Petri dishes* (Experiment 48):

*Reaction of controls*—"4++". Pustules orange instead of reddish brown. (This color difference was associated with high atmospheric moisture content, a relation that has been observed by other investigators).

*Effect on infection type of continuous darkness for: 1 day*—little effect. *Two days*—development retarded, final reaction "4+" on slightly pale leaves. *Three days*—type-"3" pustules in green areas on pale leaves (on 18th day after inoculation). *Four to 9 days*—gradation from pale green to white to brown leaves, as time in dark increased, all with green areas surrounding rust pustules, which were progressively smaller and paler as the time of continuous darkness increased. On none of the leaves did any hypersensitive tissue appear.

*Effect on host tissue of continuous darkness for: 2 days*—leaves slightly pale. *Three days*—leaves pale. *Four days*—leaf bases nearly white, tips pale green. *Five days*—bases nearly white, remainder of leaves fairly uniformly pale. *Six days*—almost completely etiolated. *Seven to 9 days*—completely etiolated, partially injected, 1 leaf brownish.

*Seedlings in pots* (Experiment 47):

*Reaction of controls*—"4++".

*Effect on infection type of continuous darkness for: 6 days*—"x", necrotic flecks closely resembling Khapli type accompanying some pustules. Teliospores in some small pustules.

*Effect on host tissue of continuous darkness for: 6 days*—very slightly pale.

LITTLE CLUB, EXPERIMENT 96 (not illustrated)—30 pots planted Oct. 28, 1930, 20 pots inoculated Nov. 6, by spreading spores. To dark chamber Nov. 14.

*Detached leaves with cut ends in water:*

*Reaction of controls*—"4".

*Effect on infection type of continuous darkness for: 1 day*—unchanged. *Two to 5 days*—(detailed notes kept only up to 2 weeks from inoculation). Pustules somewhat smaller, but even on 5-day leaves rust development kept pace with that on 1-day attached leaves. Pustules surrounded by green areas on progressively paler leaves. No hypersensitive tissue at any time.

*Effect on host tissue of continuous darkness for: 1, 2 days*—no change. *Three days*—slightly pale, except the tips. *Four days*—tips very slightly pale, bases quite pale. *Five days*—leaves pale, paler at the base than at the tip, none flaccid or with dead areas, but paler than the corresponding leaves of the potted plants.

*Seedlings in pots:*

*Reaction of controls*—"4".

*Effect on infection type of continuous darkness for: 1 to 5 days*—(detailed notes kept until 2 weeks from inoculation). Rust development much slower than on detached leaves receiving corresponding treatment. Hypersensitive flecks developed on plants 3 days or longer in dark.

*Effect on host tissue of continuous darkness for: 1 day*—no effect. *Two days*—upper portions slightly pale. *Three days*—slightly pale with tips pale. *Four days*—upper quarter of most leaves pale, slightly pale below this, several tips dead. *Five days*—upper halves of leaves very pale, most leaves not fully turgid, several tips dead, a few leaves with the upper quarter dead.

*Recapitulation.* It is evident that detached leaves kept in the dark present an entirely different picture from that offered by whole plants in pots. This divergence is expressed in a different sequence of changes in appearance as well as in differences in respiratory behavior. Associated with these indices of a modified dark metabolism following detachment was a difference in rust behavior, the essential feature of which was the elimination of any development of hypersensitive tissue after prolonged periods of darkness. Such relations should prove significant ultimately in directing our search for those physiological processes responsible for the causation of compatibility or incompatibility of the host with a physiologic form of rust.

## GENERAL DISCUSSION

In interpreting the experimental results described in this paper, the possible importance of the catabolic phases of host metabolism has been given an emphasis that has not previously been accorded to it in considerations of the effect upon rust infection of modifying host metabolism by darkness. As far as concerns abundance and rate of growth of the rust, there is nothing in the results presented to indicate dependence of the rust on products of synthesis rather than of catabolism, and, as far as concerns the determination of compatible or incompatible relations between host and fungus, the evidence all points to a direct relation not with those processes

dependent on light but with some state reached by those mechanisms remaining active when light is excluded.

My observations do not find an acceptable explanation in the theory hitherto advanced of the effect of darkness upon rust growth, wherein it has been suggested that because of the lack of some substance formed by the host only in the light rust growth ceases in darkness but is promptly resumed when photosynthesis again begins. Such a theory took origin in the equivalence discovered by Fromme (8) and Mains (19) to exist under certain circumstances between the time of continuous sojourn of infected plants in darkness and the time of retardation occasioned in the appearance of rust pustules. But, it is evident from the results here presented that such a relation is not a general one. The tendency was, instead, under the conditions of my experiments, for the period of retardation in pustule development to exceed that of subjection to darkness. This relationship suggests dependence not merely on the cessation in the dark of some process recommenced on return to light but rather on the modification of a physiological state during darkness that was reestablished on return of the plants to light. Furthermore, it frequently happened that rust pustules actually formed and broke through the epidermis of leaves while they were still in the dark chamber. The fungus was, therefore, developing under the influence of the characteristic metabolic sequence of the tissues in darkness, but it developed more slowly than in light and with very different results to the host tissues. Final proof that rust development was not simply arrested in the dark, proceeding in its usual fashion on return to light, exists in the appearance of the insignia of incompatibility following the subjection of congenial host plants to sufficient periods of continuous darkness.

The change from compatibility to incompatibility of host tissues to the rust form employed was not related to the absolute duration of darkness, as it should have been had it been solely dependent on lack of light and, therefore, on cessation of the processes to which light is essential. It was related rather to a duration of darkness, which showed an inverse relation to temperature, and was correlated with the rapidity of metabolic change in the tissues in the dark, as judged by the time required for the progression of dark metabolism to the point of death of the tissues. Under such circumstances it must be the processes remaining active in the dark, not those extinguished by the absence of light, that are important to the observed change in host-fungus relationships.

This is further substantiated by the fact that the change from compatibility to incompatibility was not related to the total dark experience of the seedlings. Interruption of darkness by short intervals of light once in 24 hours prevented the occurrence of well-defined hypersensitive tissue, while comparable seedlings, simultaneously subjected to a shorter total dark

experience so arranged that they received one long period of continuous darkness, bore sharply hypersensitive tissue.

The evidence as a whole can be very well rationalized on the assumption that disturbance of compatibility between host and fungus, as expressed in the appearance of the symptoms of resistance on hosts susceptible to the rust form involved, was consequent upon the establishment of some definite metabolic state in the host cells resulting from subjection of the seedlings to prolonged darkness under the conditions prevailing during these experiments. It is not intended to discuss in physiological terms the possible nature of the metabolic state involved until such time as the results of the researches into its nature now in progress are communicated. For a general conception of the physiological background of these investigations the reader has already been referred to literature on the dark metabolism of various plant organs (1–7), (12), (14), (18), (22), (23), (27).

In terms of this hypothesis, it is assumed that when wheat seedlings of varieties congenial to infection by *Puccinia graminis tritici* p.f.21 were subjected to darkness, they entered upon a definite course of metabolic change, which, if allowed to proceed long enough, led them to a physiological state uncongenial to rust development. The visible result was the appearance of hypersensitive tissue in the presence of fungus mycelium together with the retardation and inhibition of rust development.

After the return of seedlings to light, unless either the host tissues or the fungus mycelium had become irreversibly affected, the fungus was able to develop secondarily in the manner characteristic of the rust form in a congenial host. This may be assumed to be associated with a redirection of metabolism such as is known to occur on the return to light of certain green plant tissues after a period of darkness insufficiently prolonged to bring about a state of irreversibility (1). Once the right metabolic state had been reached, however, hypersensitive tissue developed, even though the plant was returned to light, and the secondary pustules developed under the influence of the redirected metabolism appeared not in the immediate center of primary infection but in the adjacent tissue. If, on the other hand, dark metabolism was not allowed to progress to the hypothetical initial point for production of hypersensitive tissue, but was interrupted at an early stage by a period of light sufficient to restore the original metabolic condition, repeated subjection to such short periods of darkness did not cause a serious disadjustment of compatible relations, so long as the plants were not held continuously so long in the dark that the critical metabolic state, resulting in incompatibility, was reached. This would explain the fact that in one experiment infected plants suffered nearly 300 hours of darkness, so allotted that 4 hours of light always succeeded 20 hours of darkness, without showing symptoms of uncongeniality to rust development or any

indications of retardation of rust growth; whereas, on corresponding plants receiving less than 200 hours of darkness but with a continuous period of about 60 hours, a resistant infection type resulted. Shorter daily exposure to light than 4 hours eliminated hypersensitiveness but did not maintain quite the same metabolic conditions as did the longer light periods. The fungus was retarded in its development and was unable to form pustules of normal size, and the host tissues eventually became pale and flaccid and finally succumbed. The failure of hypersensitive tissue to appear under these circumstances indicates a failure of the tissues to reach the metabolic state requisite for the replacement of compatible by incompatible relations and provides additional evidence on which to base investigations into the nature of this hypothetical metabolic state.

In different experiments, where plants differing sometimes in physiological state were also subjected to darkness at different temperatures, the rapidity of progression of the seedlings through their succession of visible changes was affected, and this is taken as an indication of similar effects on the rapidity of metabolic change. The strongly suggested correlation between this rapidity of change and the duration of darkness necessary under any set of conditions for the production of hypersensitive tissue again implies that the disadjustment of compatible relations between rust and host tissues was dependent on the establishment of some metabolic state that appeared in the course of dark metabolism. The slight differences in the duration of darkness required to bring about a change from compatibility to incompatibility, observed in seedlings differing slightly in age, could well be assigned to divergences in rapidity of metabolic change in the dark, resulting from differences in physiological state of the seedlings at the time of discontinuing illumination. That the time relations and the course of dark metabolism may be influenced by the physiological state of the tissues at its initiation is no new idea to students of dark metabolism. Blackman (2), Blackman and Parija (3), Parija (22), and Godwin and Bishop (12) have called attention to the importance of this consideration.

The experiments on detached leaves support the metabolic hypothesis. The course of visible change in darkened detached leaves, distinct from that of attached leaves, indicates a different course of metabolism in these leaves. This view is corroborated by comparative experiments on the respiration of attached and detached leaves, which revealed the fact that dark metabolism in the two differs in certain important respects. The metabolic course in detached leaves failed to establish the condition essential to the appearance of hypersensitive tissue in infected areas and allowed better and more rapid development of uredinia than occurred on corresponding leaves attached to their plants. This is seen as an important guide in a search for

the metabolic state assumed to be responsible for loss of congeniality toward the rust.

The nature of the critical metabolic state cannot be indicated as a result of the experiments described. It might involve measurable concentrations of metabolites, or protoplasmic states, or it might involve both. But the experiments reported here suggest very strongly that the physiological processes of the host that are not discontinued when light is excluded are concerned in the determination of the congeniality or uncongeniality of a host to a rust pathogen. In thus giving prominence to the processes of dark metabolism, they open up a new field for investigation into the nature of the rôle played by host physiology in the determination of rust-infection types.

These researches are being carried on in the Department of Botany, at the University of Toronto, under the direction of Dr. D. L. Bailey and Dr. G. H. Duff, to whom I am indebted for constant interest in the investigation and for invaluable criticism and advice, both in the design of experimentation and in the preparation of manuscript. The investigation has been carried on during tenure of a fellowship of the Ontario Research Foundation and research and teaching assistantships in the Department of Botany. I wish to acknowledge my indebtedness to Dr. M. Newton, of the Dominion Rust Research Laboratory at Winnipeg, for seed of the differential wheat varieties and for a culture of *Puccinia graminis tritici* p.f.21.

## SUMMARY

1. Subjection of seedlings of 11 wheat varieties to darkness for periods of from 1 to 7 days resulted in changes in the appearance of the leaf tissues; in the infection type produced by *Puccinia graminis tritici* p.f.21; in the rate of development of the rust; and, in some instances, especially with resistant hosts, in the number of pustules and visible infection centers.

2. Rust development was retarded by darkness, but the retardation was not generally equivalent to the period of darkness. The period of retardation most frequently exceeded the period of darkness.

3. The modification of infection type on seedlings subjected to prolonged periods of darkness was toward a more resistant type. It involved diminution in size of pustules or elimination of pustules, and appearance of sharply hypersensitive tisssue on ordinarily congenial hosts.

4. Interruption of darkness by short daily periods of light prevented appearance of the sharply hypersensitive tissue resulting from subjection to continuous darkness.

5. Detachment of leaves at the time of subjection to continuous darkness eliminated the sharply hypersensitive tissue resulting from subjection of infected seedlings to darkness. The effects on the general appearance of

leaf tissues were also different from those on similarly treated attached leaves.

6. Evidence was discovered of a correlation between durations of continuous darkness, required under various circumstances to cause appearance of hypersensitive tissue on seedlings, and rapidity of metabolic change in host tissue in the dark, as judged by the duration of darkness required under any set of circumstances to affect the tissue irreversibly.

7. On tissues of susceptible seedlings not irreversibly affected by darkening, secondary pustules of a susceptible type appeared in tissue adjacent to resistant primary infections resulting from darkening.

8. An hypothesis has been developed concerning the relation of modification of the infection type to the dark metabolism of the host, which offers an explanation of the results obtained and forms a basis for further experimentation.

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N. B. Approximately one-third of the cost of printing this contribution was borne by the author.

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# A ROT OF APPLE FRUIT CAUSED BY PHYTOMONAS MELOPHTHORA, N. SP., FOLLOWING INVASION BY THE APPLE MAGGOT[1]

T. C. Allen and A. J. Riker

## INTRODUCTION

Bacteria, producing rot of apple, have been isolated during the course of a study on the apple maggot, *Rhagoletis pomonella* Walsh, and an associated decay. These bacteria were first obtained by the writers from decaying apple tissue infested with the larval or "railroad-worm" stage of the apple maggot. Further isolations of these bacteria were obtained from other decaying maggot-infested fruits, from maggots, and from adult insects. All these strains appeared to possess similar cultural characteristics. Inoculations with them upon apple tissue showed them to be pathogenic. Since the losses due to this rot are quite serious to fruit growers in the Kickapoo-Valley section of Wisconsin and since so little information on this decay or its causal organism could be found in literature, further study of this problem was deemed advisable.

## ECONOMIC IMPORTANCE

The economic importance of the rot in maggot-infested apples under some conditions is considerable. Harvey (6) notes, "As the larvae grow and the fruit matures, the enlarged channels do not heal, but turn brown and the presence of the maggots is then readily detected. These channels meander through the whole fruit even to the core. They often cross each other, enlarge as the larvae grow, and in last stages of Trypeta work, run together producing large cavities. Finally they involve the whole fruit. . . . " Porter (11) illustrates the injury caused by rot within maggot-infested fruit: "Everyone living in the country, in New England and near-by states, is familiar with the disappointment experienced on biting into a 'railroaded' or maggoty apple. Many such apples give no external warning that anything is wrong, although their interior may be a broken-down mass of rotten pulp." Further mention is made of damage to apple tissue ". . . caused by the maggots, which, in feeding and moving through the fruit, break down the pulp, leaving brown trails of rotten tissue."

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. These studies have been carried on in cooperation between the Departments of Economic Entomology and Plant Pathology.

The writers wish to express their appreciation to Professors Baldwin and Frost for laboratory facilities and advice and to Professor Wilson and to Professor Granovsky for the inception of the problem and for suggestions during the earlier stages of the work.

The insidious destruction following the invasion of this maggot was emphasized by Chapman (4): "I feel rather certain that growers have been in a large number of cases unaware of the severity of the pest in their plantings. Winter fruit especially may appear perfectly sound externally at harvest to one unfamiliar with egg punctures, made earlier by the apple maggot fly, only to break down and become worthless some weeks later when it comes into the possession (often several persons removed) of the purchaser."

Decomposition in a 60-bushel lot of maggot-infested Dudley apples, held in the packing house about a week after picking, was witnessed by the senior writer during the summer of 1930. At the end of 10 days the damage due to decay and collapse of the fruit about conspicuous brown trails in the apples was so great that re-sorting was necessary. Twenty bushels of this lot were found worthless. The remaining 40 bushels appeared healthy and were therefore kept for sale to local consumers. An inspection of these apples after another week, however, showed that many had developed symptoms similar to those encountered before. Twenty-two of these 40 bushels were sorted out as healthy during a second overhauling. Later, all of them were lost because of tunneling of maggots and apple decay.

### ASSOCIATION OF THE APPLE MAGGOT WITH DECAY

The association of the apple maggot with a decay in the apple has been frequently observed by those familiar with this insect. Riley (12) associates a rot in apples infested with this maggot that, ". . . burrows in all directions, and in varied numbers, in the flesh of the fruit, giving it a discolored, honey-combed, rotten, and filthy appearance." Illingworth (7) has noted in certain maggot-infested apples, ". . . a black rot that leaves the fruit a dry, hardened mass." Porter (11) wrote: "If several maggots are present in one apple the whole interior finally breaks down, and the apple becomes a worthless mass of rotten pulp, although it may still appear perfect externally. This injury is very characteristic, and there is little danger of confusing it with that caused by any other insect." Patch (9) reports: "The apple maggot works in soft discolored mushy trails anywhere in the pulp of the apple." Pettit (10) observes that "The apple maggot bores indiscriminately through the flesh, piercing a tunnel which is of small bore for the greater part of its length, although toward the last the tunnel may, and often does, develop into large blackened cavities, excavated near or in the core." Granovsky (5) states: "Many infested apples give no external sign, having a quite healthy appearance, although their interior may be a broken-down mass of rotten pulp. The apple maggot in addition carries a bacterial organism which causes and hastens decay of apples in cold storage." A bacterial rot that commonly accompanies the work of the

maggot has been found during the past 2 years at Gays Mills, Wisconsin, as already mentioned in a preliminary report (Allen, 1).

The insect in its various stages seems to introduce the rot-producing bacteria into the apple tissue and to hasten its dissemination throughout the fruit.

Two stages of the insect, the adult and the larvae, appear to assist the distribution of these bacteria. The adult or fly seems to provide opportunity for the dissemination and entrance of the bacteria into the host. The entrance of the bacteria into the apple is apparently accomplished with the deposition of the egg beneath the cuticle. That rapid translocation of the bacteria in the fruit as associated with the maggot is shown by the rot of the apple tissue adjacent to the maggot tunnels. This apparent relation between the pathogenic bacteria and the insect suggests biologic transmission of the bacteria similar to that found by Leach (8) in his studies of the potato-black-leg disease. The evidence available on the association between these maggots and the bacteria is considered in detail with the isolation studies of the bacteria.

The purposes of the present paper are primarily to describe the symptoms of this apple rot, to present evidence on the pathogenicity of the causal bacteria, and to give the results of studies on their bacteriological characters.

## SYMPTOMS OF DECAY

The symptoms of this disease were similar (1) under natural conditions both in the orchard and in storage and (2) following inoculations under artificial conditions.

In the orchard the rot of the apple tissue may be described from the deposition of the eggs by the insect (Fig. 1, A and C). Maggots that emerged from eggs under the skin in the cheek of the comparatively green apple left minute light brown burrows (Fig. 1, B) that may often be traced from the feeding larvae to the empty eggshell. When such apples fall to the ground, the development of the feeding larvae is greatly hastened and the burrows become larger and darker brown in color, chiefly due to the advancement of decay in the tissue adjacent to their tunnels. The young larvae generally fed and bored their way toward the core. In the course of their development in this area a comparatively firm rot usually appeared about the core of the apple (Fig. 1, E, F, and G). In most cases few or no outward symptoms of this decay were yet apparent. As the apples ripen the dark brown tunnels left by the feeding larvae would often progress to the outer portions and could be seen from the surface of the fruit (Fig. 1, D). Such fruit was commonly found on the ground in infested orchards after apple harvest. The development of the rot in the orchard paralleled in many respects that found in storage.

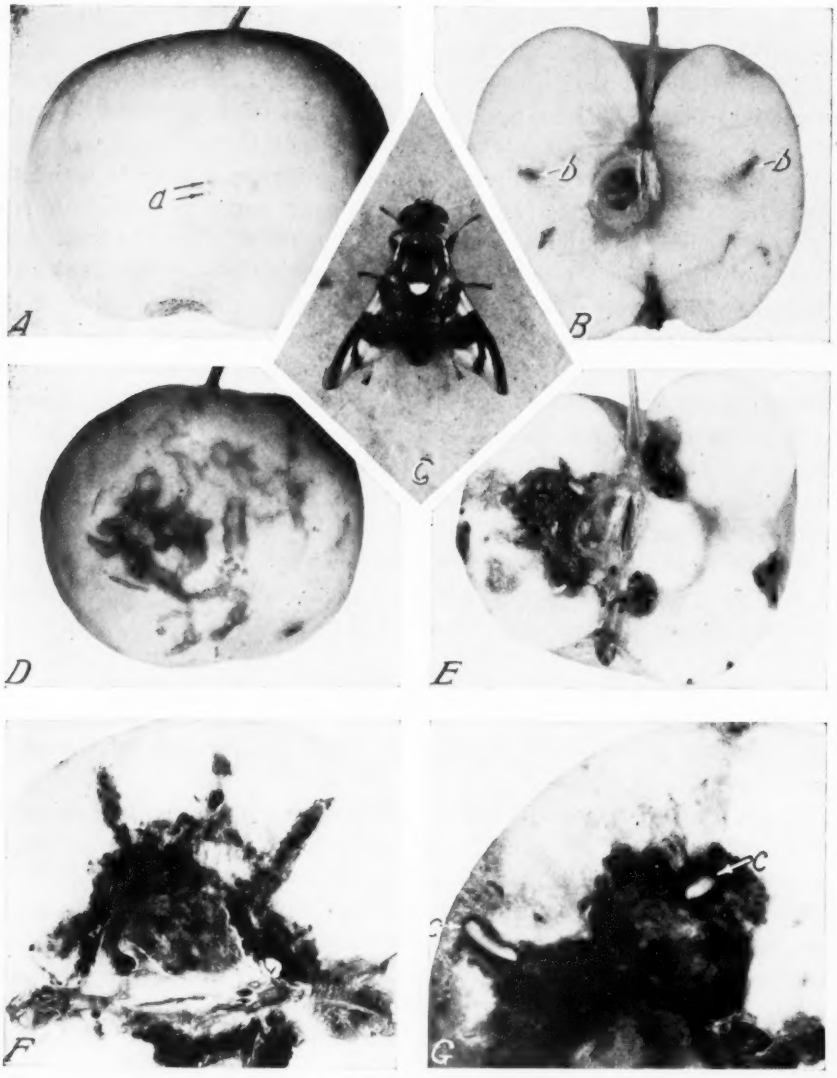


FIG. 1. A. Egg punctures in the surface of an apple, *a*. B. Burrows, *b*, of young larvae in apple tissue. C. The adult female of *Rhagoletis pomonella*. ×3. D. Burrows made by apple maggots under the skin of an apple showing early stages of decay. E and F. Section of an apple showing maggot injury and subsequent bacterial decay. G. Later stage than F. Two larvae, *c*, appear near the margin of the decaying tissue.

In storage, the early symptoms of the disease following the inconspicuous egg puncture and larval burrow occurred as internal decay within and about the core of maggot-infested apples. The line of demarcation between the

healthy outer portion of the fruit was rather sharply delimited from the decayed inner portion. Such symptoms within the fruit have been commonly noted in many early and late varieties of apples. As infested fruit became fully ripened, larvae that were feeding about the core of the apple tunneled to the outer portions of the fruit.

The common later symptoms in storage appeared as brown rot adjacent to insect burrows near the surface of the fruit (Fig. 1, D). Under favorable conditions the centers of decay rapidly advanced from these burrows into the surrounding healthy tissue. Fruits showing these symptoms were easily crushed and uninvaded portions of the healthy tissue could be readily separated from the invaded tissue. Before total destruction of the fruit occurred, the maggots bored their way out of the apples through a conspicuous exit hole. This hole often became surrounded by an area of decaying tissue. Further progress of the rot turned the entire apple into a comparatively firm but shrunken mass of decayed tissue.

Symptoms of the disease produced upon entire apples and apple plugs by inoculation from pure cultures of the bacteria were similar to those found in apples under natural conditions. Inoculations were made on apples surface-sterilized with bichloride of mercury, 1 to 1,000. Checks were made by pricking the surface of the apple opposite the point of inoculation. On entire apples, the first symptom of decay in tissue around the point of inoculation appeared as a small, light brown, slightly sunken area (Fig. 2, A). As this decaying area enlarged, it was preceded by a very narrow water-soaked halo. The rot became darker brown in color as it aged (Fig. 2, B and C). Concentric rings sometimes appeared in the surface of the diseased tissue. The epidermis of the apple seemed comparatively firm, leathery, and slightly sunken. Within the apple, the decay progressed in all directions (Fig. 2, E) from the point of inoculation, and the advancing margin of the rot could be rather sharply distinguished from the healthy tissues. As the fruit ripened it seemed to become more favorable for the growth of the bacteria, for the decay was more rapid. Entirely decayed fruit was irregular in shape, shrunken, and dark brown (Fig. 2, D). The tissue remained rather firm.

Plugs for inoculation studies were made from fresh apple tissue, with aseptic precautions, in the usual manner. These plugs varied in size and shape, but were generally $\frac{1}{2}$ to $\frac{3}{4}$ in. square and $\frac{1}{4}$ in. thick. Cylindrical plugs also were obtained by using a sterilized $\frac{5}{8}$ in. cork borer. The freshly cut tissue was immediately placed on agar previously plated in Petri dishes, inoculated, and then incubated at a temperature between 18 to 22° C.

The first symptom of the disease following inoculation of apple plugs, was usually a small brown circular region about the needle prick. This invaded portion became darker, with a narrow water-soaked margin, and

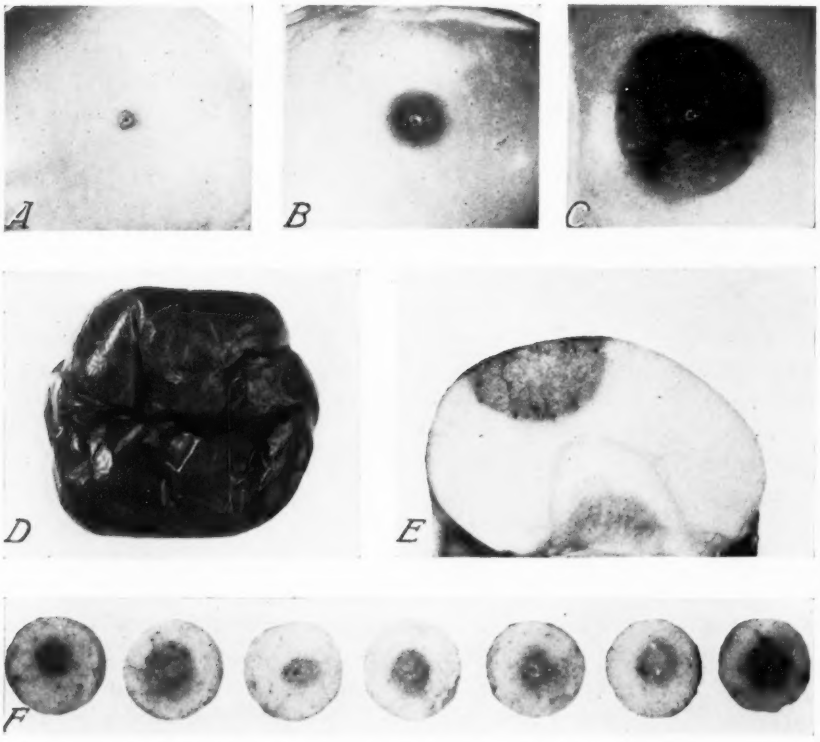


FIG. 2. A. Rot produced 2 days after inoculation about a needle puncture in a Pearmain apple. B. As A, except 4 days after inoculation. C. As A, except 16 days after inoculation. D. Entirely decayed apple several weeks after inoculation. E. Section of an apple showing decay 16 days after inoculation. F. Discs made from apple tissue showing decay about needle punctures 2 days after inoculation with the 7 single-cell cultures of bacteria from left to right, respectively.

advanced from the point of inoculation as the organism invaded adjacent tissue. A conspicuous brown area was produced by the bacteria in 24 hours. In early stages of rot, the apple tissue remained moist and often a surface ooze containing great numbers of bacteria appeared about the needle prick. Several days were generally required for the entire plug to decay. At this time the organism had penetrated the apple tissue and formed a colony on the agar. Plugs completely rotted appeared brown and later dried down. Separate plugs in each Petri dish were used as checks by pricking them with sterile needles.

### ISOLATION OF BACTERIA

Seven strains of bacteria were isolated from maggot-infested apple tissue and from different stages of the insect. The bacterial strains under con-

sideration were obtained during the fall of 1929 and during 1930 from different varieties of apples and from 2 localities in Wisconsin. Geographically, strains Nos. 1, 2, 3, and 4 were secured from Gays Mills, Wisconsin, and strains 5, 6, and 7 from Fort Atkinson, Wisconsin.

Strain No. 1 was secured from isolations made from young insect larvae transferred from beginning stages of rot in a Wealthy apple.

Strain No. 2 was secured from isolations made from advanced margins of decay about larval burrows in Snow apples.

Strain No. 3 was secured from the surface of an adult apple-maggot fly caught in the orchard.

Strain No. 4 was secured from the dissected abdomen of a female adult apple-maggot fly. The abdomen of the fly was seared before making an incision and a portion of the alimentary canal was withdrawn and placed on culture media.

Strain No. 5 was secured from larval burrows in a Greening apple that was in the early stage of decay.

Strain No. 6 was secured from larval burrows in an unnamed sweet apple.

Strain No. 7 was secured from the surface of a young larva that was producing brown trails of decayed tissue in a Greening apple.

Isolations of the organism from invaded apple tissue were accomplished with the usual laboratory technique. Before such isolations were made the apples were surface-sterilized with bichloride of mercury 1 to 1,000 and the fruit cut or broken open with a sterile scalpel. In isolating the bacteria from the apple proper, portions of the recently invaded tissue were transferred either to water blanks or melted agar, macerated, and used in dilution plates of nutrient glucose agar. In isolating the bacteria from the surfaces of the larvae, the maggots were removed from the diseased tissue and placed on agar in Petri dishes. Individual colonies of the bacterium were usually obtained from the plates following a period of 2 or 3 days. Approximately 20° C. was a satisfactory incubation temperature.

Numerous yeasts as well as bacteria appeared on the poured plates. Attempts to produce rot by inoculations into apple with the yeasts alone all gave negative results. To discourage these yeasts, crystal violet (1 to 200,000) was added in some series to the nutrient glucose agar.

The bacterial cultures were purified as described later.

### PATHOGENICITY

The pathogenicity of the bacteria under consideration was determined by successive isolations and inoculations. Two series of inoculations were made with each of the 7 purified strains of bacteria on Wealthy apple tissue. Each strain was inoculated in duplicate into apple plugs and also in dupli-

cate into entire apples. Unless otherwise stated, the inoculated apple tissue was kept at room temperature. The plugs from Wealthy apples were prepared under aseptic conditions and placed in Petri dishes on 10 cc. of agar intended to maintain the moisture. Three plugs were distributed in each of 7 Petri dishes with the usual precautions to prevent contamination. A strain of the bacteria was inoculated, respectively, with a needle into 2 of the plugs in a dish. The remaining noninoculated plug was pricked by a sterile needle and used as a check. The water-soaked halo appeared in about 12 hours. In every case the rot was conspicuous in 2 days (Fig. 2, F) and usually involved the whole plug in a week. No rot developed in the controls.

Reisolations of the different strains of apple-rot bacteria were secured from the above series of inoculations made into apple plugs. Small sections were taken from the most advanced portion of decaying tissue and macerated in Petri dishes. Subsequently dilutions were made and colonies of the bacteria appeared in the poured plates that resemble the original cultures of the apple-rot organism.

Reinoculations of the different strains of bacteria secured from these reisolations were made into apple plugs. The technique employed in this work was identical to that already described in the first series of inoculations of the bacteria on apple plugs. Rot was produced after 48 hours about the needle pricks of the reinoculated cultures. No rot developed in the control plugs.

Entire Wealthy apples also were used to test the pathogenicity of each of the 7 bacterial strains. Before inoculations were made the apples were washed and surface-sterilized with bichloride of mercury, 1 to 1,000. One apple was used for each strain. The apples, respectively, were then inoculated twice with each strain of the bacteria. Two sterile needle punctures were made as controls. The apples were placed under sterilized inverted beakers at room temperature. The first symptoms became apparent about the inoculations within 2 days (Fig. 2, A). After this time the rot became progressively more and more conspicuous (Fig. 2, B, C, D, and E). No rot developed about the control punctures.

Reisolations of the different strains of apple-rot organism were secured from the above series of inoculations made into entire apples. The epidermis of the apple above the most advanced area of decay was removed and a small portion of the rotted tissue was transferred to Petri dishes and macerated. Subsequent dilutions and poured plates were then made. Colonies that resembled the original cultures appeared in 48 hours in the agar of the poured plates.

Reinoculations of the different strains of bacteria obtained from these reisolations were made into entire apples. The technique employed in this

work was a repetition of that already described in the first series of inoculations of the bacteria on entire apples. Rot was produced in a few days about the needle pricks of the reinoculated cultures.

Additional inoculations were made either into entire apples or apple plugs made from Delicious, Duchess, Dudley, McMahon, McIntosh, Patten's Greening, Rome, Snow, and an early sweet russet apple. Rot was produced in all the trials made with these apples.

Susceptibility of different varieties of apples or related fruits to invasion by this bacterial organism has not been studied. It was noted, however, that in some apples many weeks were required for the organism to rot the entire fruit. This was observed in more firm varieties, as Northwestern Greening, and in comparatively mellow varieties, such as McMahon. On the other hand, such varieties as the early sweet russet, Delicious, Wealthy, and Dudley, which are sweet and mellow, decayed more readily. These limited observations appear in correlation with Porter's (11) statement that the apple maggot seems to be attracted to sweet apples in which the flesh is more easily broken down by the larvae.

Green apples appeared less susceptible to rot than ripe apples in experiments on Wealthy, McIntosh, and Northwestern Greening. Two apples of each variety were inoculated through needle punctures in 3 places of each apple. Equally numerous control punctures were made. Little or no rot followed these inoculations. These apples were picked from the trees 2 weeks following the inoculations and held in storage for observations, but no rot developed. Parallel inoculations and control punctures were made into apples of the same varieties a few days after they had been picked. The inoculated fruits generally developed considerable decay within a suitable incubation period.

## BACTERIOLOGICAL STUDIES

A favorable medium on which to grow the bacteria during the isolation and inoculation studies was sought in preliminary studies. Tests on different media showed very little growth by the bacteria in beef-extract and Bacto-peptone broths unless a sugar were present. As described later in this paper, the most vegetative growth was made in media containing glucose. Nutrient glucose agar was found satisfactory for this isolation work.

Separation of the 7 different bacterial strains previously mentioned from yeasts and other organisms was tried by several successive replatings with the usual technique. The medium employed was nutrient glucose agar. The dilution plates were incubated at room temperature for a period of 2 or more days. Individual colonies were picked from dilution plates in which fewer than 60 individual colonies appeared per plate. Additional

series of plates were poured if the growth suggested the presence of an undesired organism in the culture.

The technique employed in the bacteriological studies during the fall of 1930, unless otherwise noted, was that given by the Committee of Bacteriological Technique of the Society of American Bacteriologists (13). Each of the tests was run in duplicate with suitable controls. These duplicate tests were made in most cases 3 different times for each of the 7 strains of bacteria under consideration. All the determinations for an individual character were similar, except as noted, for each of the 7 different strains. Unless otherwise mentioned, the cultures were incubated at approximately 21° C.

### MORPHOLOGICAL STUDIES

The shape and size of the bacteria were determined from slides made from 18-hour-old cultures on nutrient glucose agar and stained with Gram's stain. The vegetative cells observed were short rods, with round ends arranged singly or in pairs, and at times in short chains. A hundred measurements with a filar micrometer showed that the bacteria were 0.6 to 1.0 by 0.8 to 1.9 μ in size, with the greatest number about 1.32 by 0.68 μ.

Motility was observed shortly after transfers began to grow. After the cultures were from 18 to 24 hours old, however, their movement diminished. Motility was demonstrated in hanging-drop cultures and in suspensions mixed with India ink.

Flagella stains showed that the bacteria possessed lophotricheous flagella. Strains Nos. 1 and 2 only were used for staining flagella. With Casares-Gils' flagella stains the writers were able to observed 2 polar flagella. Mr. H. E. Sagen, however, demonstrated still more clearly the presence of 2 polar flaglla with Loeffler's, Gray's, Casares-Gils', and Bailey's (2) method of staining flagella.

Young cultures of the apple-rot bacterium were Gram-negative, but with age Gram-positive cells appeared. Considerable variation of the bacteria to Gram's stain was at first noted in 2-day- to 6-day-old cultures. As a check on the technique (Stitt, 14), the apple-rot organisms were always stained on slides between smears of Gram-positive and Gram-negative organisms. Cultures of the following ages were employed: 12, 16, 20, 24, 28, and 36 hours, 2, 4, 8, 16, and 32 days. It was found that, while all strains of the apple-rot organisms less than 24 hours old gave consistent Gram-negative results, distinct Gram-positive organisms appeared to increase in number in the older cultures.

Capsules have not been demonstrated. Efforts were made to find them by examining suspensions of 18-hour-old cultures in India ink and by staining with Hiss's and with Gin's capsule stain. India-ink preparations

were made on slides by adding a loop of India ink to a suspension of motile bacteria.

Tests for the formation of spores were negative. Thirty-day-old cultures were stained according to the Dorner and Ziehl-Neelsen methods, but no spores were detected. Thirty-day-old cultures, grown on nutrient agar, also were heated 12 minutes at 85° C. and transferred to nutrient glucose agar. No growth appeared.

### GROWTH CHARACTERS ON VARIOUS MEDIA

The colony characters were determined on nutrient agar containing 2 per cent of glucose. An incubation temperature of about 20° C. was maintained for all these studies. The bacterial colonies appeared on the medium after 36 hours as minute circular, almost transparent, growths. After 3 days they appeared as follows: Surface type: growth, rapid; form, circular; surface, smooth, glistening; elevation, convex; edge, smooth, entire; internal structure, finely granular; optical characters, opaque; chromogenesis, light pink. This color usually appeared in 4 to 7 days. The intensity of color and the time at which this chromogenic character appeared varied considerably. In cultures several weeks old, as the agar dried, the pink tint became somewhat brown. This chromogenic character was of considerable assistance during the earlier isolation and purification studies. It appeared, however, not always to be a constant character on different media.

Deep type: Form, lens-shape; internal structure, dense. Deeply submerged colonies made very little growth.

Agar strokes, incubated 4 days at 18° C. on nutrient glucose medium, had these characters: growth, moderate; form of growth, contoured to filiform; elevation, raised; surface, smooth; optical character, opaque; chromogenesis, usually pink; odor, absent; consistency, butyrous; medium, unchanged in color.

In gelatin stabs no liquefaction occurred and the medium was unchanged in color. A very scanty villous form of growth was generally made along the line of puncture.

In nutrient broth, with 2 per cent glucose, good growth of the organism was made in 24 hours. The medium was slightly cloudy. Sediment was formed, which was viscid on agitation. In 48 hours the amount of sediment was abundant and compact. A pellicle was formed. The broth became slightly clouded by a flocculent development that, on agitation, dispersed as a fine granular suspension.

Sugar-fermentation studies were made in nutrient broth. The organism was allowed to grow 5 days in 1 per cent glucose, saccharose, and lactose broths containing Durham tubes. Tests of the hydrogen-ion content by the colorimetric method were then made. Acid was formed from glucose and

sucrose, but very little, if any, was found in the medium containing lactose. Gas was not formed in any of the fermentation tubes. The density of pellicle and sediment showed that the growth made was more pronounced in the medium containing 1 per cent glucose. The least amount of growth was made in 1 per cent lactose.

The diastatic action of the bacteria was determined on 2 per cent starch agar. Starch was not hydrolyzed. Little growth occurred after the first 24 hours following the transfers. After an incubation period of 7 days at room temperature a saturated solution of iodine in 50 per cent alcohol was poured over the agar. No clear zone developed about streaks in any of the plates.

Reactions in milk were recorded from studies of inoculated litmus-milk cultures. Such cultures were incubated at various temperatures and were observed over a period of 6 to 8 weeks. The litmus milk showed little, if any, change.

Reduction of nitrates was tested in the usual way. Tubes of $\frac{1}{10}$ per cent of potassium nitrate broth, containing Durham tubes, were inoculated with the bacteria. The broth contained no sugar. In 7 days only a slight growth was made by the organism, which appeared as a scanty, white, finely granular sediment on the bottom of all test-tubes. No gas was formed. Tests with Trommsdorf's reagent showed that no nitrites were present. Trials with diphenylamine showed that the nitrates were still present.

No indol was found in tests made with 6-day-old cultures. A scanty, white, chalky sediment had settled on the bottom of these tubes, which indicated that only little growth had occurred. Indol tests were made according to Ehrlich-Böhme's technique.

### STUDIES OF CULTURES DERIVED FROM INDIVIDUAL CELLS

Individual cells were picked and grown from each of the 7 apple-rot organisms under consideration, so as to remove, as far as feasible, the possibility of the presence of some undesired organism. Although the writers had as much confidence in the cultures secured by repetitions of the poured-plate method as this technique justifies, the published (15) and unpublished results secured at Wisconsin by this method from supposedly pure cultures made the writers wish to check some of the earlier results with the apple-rot bacteria against cultures grown from individual cells. A number of single-cell cultures were secured through courtesy of A. A. Hendrickson, which were isolated with the double Chamber's micromanipulator and grown as recently described (15). Two single-cell cultures from each of the 7 strains under consideration were chosen for further study. They were used in a number of selected bacteriological determinations, as described in the following pages. Unless otherwise stated, all these bacteriological determinations were made in triplicate. Pathogenicity tests were

made with the single-cell cultures, on entire Delicious apples. Rot was produced by all the cultures following inoculations into entire apples, as previously described in the pathogenicity studies.

The same bacteriological characters were found for the 14 single-cell cultures as for the 7 original strains in regard to Gram's stain, nonliquefaction of gelatin, nonhydrolysis of starch, no change in litmus milk, no reduction of nitrates, and no production of indol.

Some additional determinations were made with these single-cell cultures.

The rate of growth at various temperatures was determined by measuring the diameter of bacterial colonies grown on nutrient glucose agar. Two strains of the single-cell bacteria were used, so as to make a total of 36 colonies at each temperature. Only 6 colonies were grown in a Petri dish that contained 20 cc. of agar. The loss of moisture from the agar was mostly prevented by sealing the plates with adhesive tape. The plates were incubated at each of the following temperatures: 4, 8, 13, 17, 21, 25, 28, and 32° C. In 8 days the average diameters in millimeters of the colonies were, respectively, 0.0, 0.5, 1.7, 2.7, 4.6, 5.1, 4.5, and 4.0. In 16 days they were, respectively, 0.8, 1.1, 2.6, 3.8, 7.7, 7.6, 6.6, and 6.4. The results of these studies indicate that the maximum vegetative growth of the bacteria on nutrient glucose agar is made between approximately 21 and 25° C.

Actions of the bacteria on different carbohydrates and related substances were tested in peptone-salt medium, like that employed by Wright *et al.* (15). Cultures of each strain were run in triplicate in broth containing 0.5 per cent of these substances, which were autoclaved separately and added under aseptic conditions: arabinose, glucose, galactose, sucrose, maltose, lactose, levulose, starch, dextrin, inulin, mannitol, and glycerin. When the cultures had incubated at room temperature for 7 days, the hydrogen-ion concentration was determined by the colorimetric method. The results are recorded in table 1. In accordance with the earlier tests made upon several sugars, the most growth and the largest amount of acid were produced in the glucose broth.

## DISCUSSION

The characters of this apple-rot organism do not appear similar to those given in any description that could be found for a known bacterial plant pathogen. The kind of rot produced on apple fruit along with the bacteriological characters seems to distinguish it from any plant-pathogenic bacteria that might be considered as close relatives. Consequently, in consideration of the rot it produces, this apple-rot organism is named, according to Bergey's system (3), **Phytomonas melophthora,** n. sp. This name was selected in consultation with Professor A. G. Laird. Synonyms according to other systems of classification in common use among plant

TABLE 1.—*Hydrogen-ion concentration produced by the various strains of apple-rot bacteria from selected carbohydrates and related substances after an incubation of 7 days in peptone-salt medium at room temperature*

| Substances tested | Hydrogen-ion concentration from strains number: | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Control |
| Arabinose | 4.4 | 4.4 | 4.5 | 4.4 | 4.4 | 4.4 | 4.4 | 7.0 |
| Glucose | 3.8 | 3.7 | 3.9 | 3.6 | 3.9 | 3.9 | 3.9 | 6.9 |
| Galactose | 4.2 | 4.2 | 4.2 | 4.2 | 4.2 | 4.2 | 4.2 | 6.9 |
| Sucrose | 4.4 | 4.4 | 4.5 | 4.4 | 4.5 | 4.6 | 4.6 | 6.9 |
| Maltose | 6.7 | 6.7 | 6.8 | 6.7 | 6.7 | 6.7 | 6.8 | 7.0 |
| Lactose | 6.9 | 6.9 | 6.9 | 6.9 | 6.9 | 6.9 | 7.0 | 7.0 |
| Levulose | 6.3 | 6.3 | 6.4 | 6.3 | 6.4 | 6.3 | 6.3 | 6.9 |
| Starch | 6.9 | 6.9 | 6.9 | 7.0 | 7.0 | 6.9 | 6.9 | 6.9 |
| Dextrin | 6.8 | 6.9 | 6.8 | 6.8 | 6.9 | 6.9 | 6.9 | 6.9 |
| Inulin | 6.9 | 6.9 | 6.9 | 6.9 | 6.9 | 6.9 | 6.9 | 6.9 |
| Mannitol | 6.5 | 6.5 | 6.6 | 6.5 | 6.7 | 6.7 | 6.7 | 6.9 |
| Glycerine | 5.9 | 5.9 | 5.9 | 6.0 | 6.0 | 6.0 | 6.0 | 6.9 |

pathologist, are *Pseudomonas melophthora*, n. sp., and *Bacterium melophthorum*, n. sp. A brief characterization follows:

*Phytomonas melophthora*, n. sp.

This organism was found to be a short rod, 0.8 to 1.9 μ by 0.6 to 1.0 μ, average 1.32 by 0.68 μ, motile by means of 2 polar flagella, Gram-variable and without spores. On nutrient glucose agar incubated at 20° C. for 3 days the colony characters were: growth, rapid; form, circular; surface smooth, glistening; elevation, convex; edge, smooth, entire; internal structure, finely granular; optical characters, opaque; chromogenesis, light pink. Deep colonies were lens-shape and the internal structure was dense. Gelatin was not liquefied. Little growth appeared in standard bouillon broth. Acid, but no gas, was formed from arabinose, glucose, galactose, sucrose, levulose, and glycerin. Traces of acid also were produced from maltose and mannitol. Very little, if any, acid was detected in the media containing lactose, starch, dextrin, or inulin, respectively. Starch was not hydrolyzed. Milk was practically unchanged. Nitrates were not reduced. Indol was not formed. No hydrogen sulphide was given off. The most abundant vegetative growth occurred approximately between 21 and 25° C. The bacteria have been found in association with *Rhagoletis pomonella* and have been shown to cause decay in comparatively ripe apple fruit.

## SUMMARY

1. A bacterial rot of apple fruits has been observed both in the orchard and in storage that appeared to be associated with the apple maggot.

2. Bacteria that produced rot in apple fruits have been isolated from the decaying apple tissue and from both the larvae and adults of the apple maggot.

3. Seven different isolations of these bacteria from different sources have been purified and followed through the following cycle: inoculation into apples, production of decay, reisolation, comparison with original cultures, and reinoculation into apples followed by the typical rot.

4. Bacteriological studies are described. This organism is called *Phytomonas melophthora*, n. sp.

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# THE BASIS FOR SCLEROSPORA SORGHI AS A SPECIES

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As study of the Sclerosporas progresses it becomes evident that increasing knowledge necessitates changes in the status of certain members of the genus. Of these *Scl. graminicola* var. *Andropogonis sorghi* of Kulkarni clearly demands revision.

This fungus was first mentioned by Butler in 1907 (2), when he reported the oogonial phase of a Sclerospora on *Andropogon sorghum* L. from the Bombay and Madras Presidencies, India, and, impressed by its similarity to that of *Scl. graminicola* (Sacc.) Schroet. on *Pennisetum typhoideum* L., tentatively assigned it to that species.

In 1913 Kulkarni (9) found not only the oogonial but also the conidial phase of the fungus on sorghum in the Bombay Presidency. He was impressed by the similarity in the oogonial phase, previously noted by Butler, and by the realization that on these phases specific distinctions are based in some related groups, but he noticed that the conidia germinated invariably by hyphae and never by emission of zoospores as in typical *Sclerospora graminicola* and decided that this, with other minor distinctions in the effect on the host and in the characteristics of the conidiophores and sterigmata, justified giving the fungus varietal rank as *Scl. graminicola* var. *Andropogonis sorghi*.

In 1913 Ito (8, p. 218), discussing the Sclerospora of sugar cane recently described by Miyake in Formosa, suggested separating the genus Sclerospora into Eusclerospora, with conidia germinating by zoospores, and Peronosclerospora, with conidia germinating by hyphae, and included Kulkarni's variety on sorghum in the subgenus Peronosclerospora and thus emphasized its alliance to the truly conidial oriental forms rather than to the zoosporangial type of *Scl. graminicola*. In 1918 Butler (3, p. 205), in a detailed discussion of the species of Sclerospora known from India, compared typical *Scl. graminicola* with the variety on sorghum and suggested: "It is not improbable that these differences [in germination and sterigmata] will eventually be considered of sufficient importance to constitute distinct species, but the life history and, in particular, the ability of these fungi to pass from one host to another, is not yet sufficiently known for a decision to be arrived at." In 1924 Weston (14, p. 781), after studying the conidial phase of typical *Scl. graminicola* on Setaria in the United States and comparing it with the material of the variety on sorghum kindly furnished by Dr. Butler, decided that the variety "is certainly a distinct

[1] The writers take this opportunity of thanking Mr. M. K. Desai for the ungrudging assistance rendered throughout this work.

species, one which, aside from other differences, needs only the absolute criterion that its conidia lack an apical papilla of dehiscence to distinguish it without question from *S. graminicola*. It is clearly a species closely allied to the destructive, predominantly conidial Sclerosporas of the Orient even though it is apparently connected with an oogonial stage, presumably that of *S. graminicola*." In 1928 the same writer (16, p. 937) again emphasized this point, and since then additional suggestions have been made that Kulkarni's variety is indeed of specific rank.

Moreover, biological evidence also has accumulated to substantiate this view. In 1918 it was first suggested by Butler (3, p. 223) that "In India the bajra (Pennisetum) fungus will not attack jowar [*Andropogon sorghum*] leaves and *vice versa*. It is also noticeable that the fungus in some localities in India is confined to one host, even though some of the others may be growing near by. Thus in Pusa it has only been found on *Setaria italica* though jowar and bajra have been grown in the vicinity. This suggests that the parasite has split into specialized races, each confined to one or more hosts, as in cereal rusts." In recent years the work of Uppal (10) and of Uppal and Desai (11) has given increasing evidence of the correctness of Butler's assumption.

When all these points are considered the time seems favorable for presenting in the following paper the evidence that to the writers seems a convincing basis for establishing *Sclerospora sorghi* as a distinct species.

## STRUCTURAL DISTINCTIONS

The life cycle of this fungus on sorghum regularly comprises both a conidial phase and an oosporic phase, as does that of the type species *Sclerospora graminicola* on Pennisetum and Setaria. The material for study was collected from the leaves of infected sorghum plants at Poona, India, during the period of optimum conidiophore production at night and was carefully compared with similar material of the type species *Scl. graminicola* on *P. typhoideum* from the same locality and with the United States material previously studied by Weston (14, 16). The material was also killed and preserved in alcohol or in dilute chromacetic acid and was studied and drawn either in these original preservatives, dilute glycerine, or lactophenol. The preserved material gave smaller measurements of the conidia and conidiophores than the fresh, living material on which all measurements given herein are based.

The conidiophores bear a branch system of usually 3 primary branches approximately equal in size and in extent of development, spreading out from the main axis in close succession so that the conidia borne on the tips of the branchlets lie in a hemispherical plane (Pl. I, B, 1, 2, 4), while in *Sclerospora graminicola*, as already pointed out by Weston (14), the

branches commonly are not of equal rank since usually one can trace up through the branch system a more or less definite continuation of the main axis, from which short branches grow out abruptly at irregular intervals so that the conidia lie in irregularly disposed bunches (Pl. I, A, 1, 2, 4). This distinction, although not emphasized by Kulkarni (9), is none the less brought out when his plate 7, figures 6 and 7, is compared with his figure 3.

Moreover, the sterigmata (*i.e.*, papillae of Kulkarni), the tapering, ultimate branch tips on which the conidia are borne, tend to be somewhat longer in the sorghum fungus. This was emphasized by Kulkarni as an important distinction, the average lengths being 16.3 μ for those of the fungus on sorghum as opposed to 8.3 μ for those of *Sclerospora graminicola* on Pennisetum. Although more extensive comparisons of a wider range of material show this feature to be more variable than Kulkarni supposed (*cf.* Pl. I, A and B, 2 and 4), it still remains a difference of corroborative value in distinguishing the two.

Moreover, the conidiophores of the sorghum fungus almost invariably show a transverse septum located usually about halfway between the conidiophore base and the beginning of the branch system, thus cutting off a distinct basal cell below from the main axis proper above (Pl. I, B, 1–4). The septum developing as a thick centripetally closing ring of modified wall substance is usually complete in mature conidiophores (*cf.* Pl. I, B, 3) and usually single, the presence of more than one being exceptional (Pl. I, B, 9, 10). The basal cell begins somewhat knobbed or swollen and then continues with an approximately constant diameter of 7 to 9 μ to the septum, its length being usually equal to the main axis above (*i.e.*, about 100 μ), occasionally slightly longer (Pl. I, B, 10), rarely distinctly shorter (Pl. I, B, 8).

In *Sclerospora graminicola*, on the contrary, the base may show some modification (Pl. I, A, 1, 2) in that the wall may be thicker and of slightly different composition compared with the main axis above, but only very rarely is a septum formed delimiting a recognizable basal cell (Pl. I, A, 3) (*cf.* also rare cases figured by Weston 14, Pl. 2, Fig. x and y; Weston 15, Fig. 4, j, for American material). In this species, also, the basal portion, if thus modified, usually extends less than $\frac{1}{3}$ of the total length of the upright axis, *i.e.*, around 40 μ out of 150 to 200, and usually increases rapidly in diameter from 8 to 10 or 12 μ in this distance.

The conidia themselves, however, present features most sharply distinguishing *Sclerospora sorghi*. In shape, for example, they are most commonly broadly rotund, with the greatest diameter at the middle and the ends equally bluntly rounded (Pl. I, B, 5a, 6, 7a), whereas in *Sclerospora graminicola* frequently a point somewhat above the middle shows the greatest breadth, and above this the spore is bluntly rounded, while below it

tapers gradually to a more narrowly rounded base (Pl. I, A, 5, 6, 7). In size there is relatively little difference, as was expressed in Kulkarni's measurements of 18 to 32 μ by 16 to 23 μ for the conidia on sorghum as opposed to 19 to 31 μ by 16 to 21 μ for those of *Scl. graminicola* and as is shown in the accompanying table 1, which by more adequate quantitative expression of sizes brings out the tendency to greater breadth in the conidia on sorghum. These characteristics of the conidial phase persist unaltered when the fungus develops on maize, so that, even on this host, which is in a tribe, Maydeae, separate from the Andropogoneae, to which sorghum belongs, the distinctive features are retained.

TABLE 1.—*Comparison of sizes of the conidia of Sclerospora graminicola on Pennisetum typhoideum and of Scl. sorghi on Andropogon sorghum at Poona, India*

| Length | | | Diameter | | |
|---|---|---|---|---|---|
| Classes in μ | Number of conidia in 400 | | Classes in μ | Number of conidia in 400 | |
| | *Andropogon sorghum* | *Pennisetum typhoideum* | | *Andropogon sorghum* | *Pennisetum typhoideum* |
| 13 to 14.9 | 0 | 0 | 13 to 14.9 | 0 | 22 |
| 15 to 16.9 | 6 | 15 | 15 to 16.9 | 20 | 243 |
| 17 to 18.9 | 9 | 63 | 17 to 18.9 | 29 | 82 |
| 19 to 20.9 | 98 | 182 | 19 to 20.9 | 180 | 48 |
| 21 to 22.9 | 112 | 77 | 21 to 22.9 | 113 | 5 |
| 23 to 24.9 | 132 | 47 | 23 to 24.9 | 53 | 0 |
| 25 to 26.9 | 40 | 14 | 25 to 26.9 | 5 | 0 |
| 27 to 28.9 | 3 | 2 | | | |

The foregoing distinctions between *Sclerospora graminicola* and the species on sorghum are for the most part relative rather than absolute, quantitative rather than qualitative, variable rather than constant. Their appreciation always necessitates laborious comparisons and measurements of a large number of individuals; their expression in some instances requires compilation in tabular form. If between these 2 fungi these were the only differences, taken together they would serve as distinguishing features, and one would perforce use them, as one must, in separating various species in this genus and in related genera. Fortunately, however, this is not necessary, and these differences can be relegated to the position of merely supplementing and augmenting one absolutely clear-cut, qualitative distinction.

This distinction is that in the conidia of the fungus on sorghum the wall continues unaltered and quite unmodified across the apex (Pl. I, B, 5a–7a), while, in the case of *Sclerospora graminicola*, the end of the spore at maturity is thickened into a boss-shape protrusion of modified wall substance, the apical papilla of dehiscence (Pl. I, A, 1, 5, 6, 7). This structural

feature differentiates the fungus on sorghum with absolute distinctness from *Scl. graminicola* on Pennisetum and related hosts and would indeed distinguish it if the 2 species were to be found growing together on the same leaf of sorghum. Concomitant with this distinct structural difference, there is also an absolute difference in the method of germination of the spore, for in *Scl. graminicola* the content divides into zoospores that emerge through the softened apical papilla and swarm about, while the conidia of the fungus on sorghum invariably germinate by giving rise to 1 or 2 hyphae (Pl. I, B, 7a). That this is an absolute distinction is evidenced by the fact that only rarely in hundreds of cases observed and under conditions very unfavorable for normal development have the sporangia of *Scl. graminicola* ever been seen to send forth hyphae, and then only abortive, scanty growths (*cf.* Hiura 6, Weston 16), while in the case of the species on sorghum the conidia, without exception, germinate by hyphae, if at all.

The resting-spore phase of the fungus on sorghum usually matures, as in *Sclerospora graminicola*, after the conidial phase has declined. The oogonia are formed as brown dots in the mesophyll between the fibrovascular bundles (Fig. 1, A). As the development of these spores proceeds, the tissue between the bundles is destroyed, resulting in a longitudinal splitting of the leaves along the veins into masses of tangled fibers (Fig. 1, B). This extensive destruction of the mesophyll, with the consequent shredding of the leaves, is one of the most distinctive symptoms of the downy mildew of sorghum and is also characteristic of *Scl. graminicola* on Setaria but not on *Pennisetum typhoideum*, in which case only the leaves enclosing the ears split into shreds at the tip. The spores themselves, in size, in the color and surface configuration of the thick enveloping oogonial wall, and in the diameter and wall thickness of the single oospore within, are similar to those of *Scl. graminicola*, as was originally emphasized by Butler and Kulkarni. This is further corroborated by the data on the measurements of oospores from different collections on different hosts summarized in table 2, although one of us (Weston) has found slight biometric differences in the different collections, but no absolute, clear-cut, qualitative distinctions have been brought out as yet.

The situation presented by this fungus on sorghum, then, is one of interest since in its oosporic stage it closely resembles *Sclerospora graminicola*, while in its conidial phase it is clearly distinct. The perfect or sexual stage, *i.e.*, the oosporic phase, is, to be sure, the one emphasized in certain other groups as the basis of specific distinctions, yet it should be noted that, in the closely related genera Peronospora and Plasmopara, even in such early monographs as those of Fisher (4) and of Berlese (1) specific distinctions in many cases depended on conidial phases, while more recent work of Gäumann (5), Wartenweiler (13), and others has used in the separation of

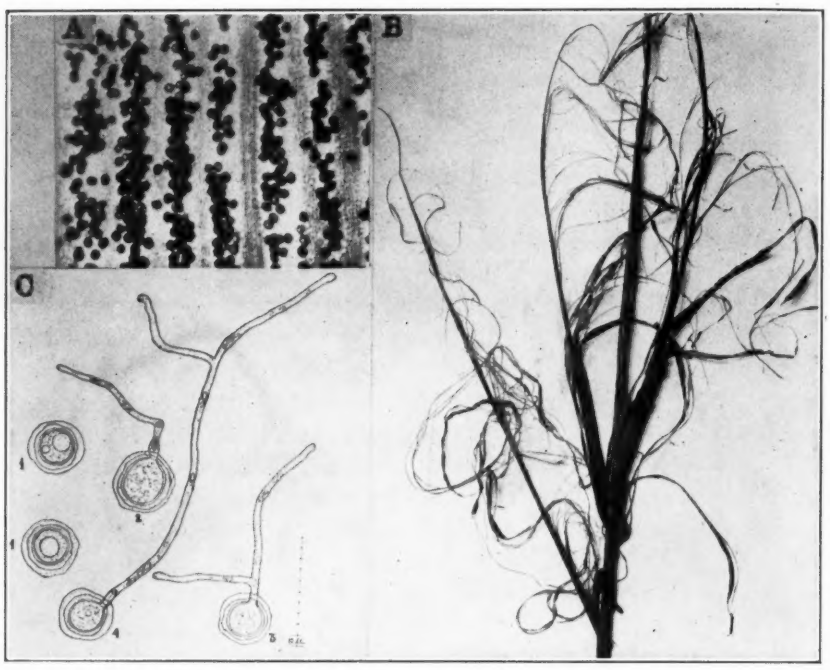


FIG. 1. A. Photomicrograph of a portion of leaf of sorghum, showing the linear arrangement of oospores in the mesophyll between the fibrovascular bundles. ×28. B. Specimen of sorghum infected by *Sclerospora sorghi*, showing the characteristic longitudinal splitting of leaves along the vascular bundles into masses of tangled fibers. ×⅓. C. 1. Resting spores, showing the single enclosed oospore. The content of the oospore is granular with 1 or 2 oil globules. 2. A germinating oospore with an unbranched germ tube. The wall of the germ tube is continuous with that of the oospore. 3 and 4. Same as 2, but the germ tube is branched. Camera-lucida drawings. Scale in microns. ×150.

species various morphological differences in the conidiophores and conidia, distinctions most of which are not absolute but decidedly relative and require expression quantitatively by large numbers of measurements of adequate populations.

When all the foregoing points are considered, the conidial phase of this Sclerospora of sorghum seems in its absolute lack of a dehiscence papilla in the apical wall of the conidia and their consequent invariable germination by hyphae, and in the definite basal cell, extensive branch system and longer sterigmata of the conidiophore, to furnish an adequate basis for its establishment as *Scl. sorghi*, a species distinct from *Scl. graminicola*.

### PHYSIOLOGICAL DISTINCTIONS

In addition to the morphological distinctions noted above, this fungus on sorghum shows certain physiological differences as well, chiefly in its

TABLE 2.—*Summarized measurements of oospores of Sclerospora sorghi and Scl. graminicola*

| Diameter | | | | | Width of wall | | |
|---|---|---|---|---|---|---|---|
| Classes in μ | *Scl. sorghi;* number in 1,000 | *Scl. graminicola;* number in 400 | | | Classes in μ | Number in 150 | |
| | | On *Setaria italica* | On *Pennisetum typhoideum* | On *Setaria viridis* | | *Scl. sorghi* | *Scl. graminicola* |
| 23 to 24.9 | 0 | 0 | 0 | 0 | 0.3 to 1.09 | 14 | 15 |
| 25 to 26.9 | 3 | 3 | 5 | 3 | 1.1 to 1.89 | 54 | 38 |
| 27 to 28.9 | 9 | 3 | 10 | 8 | 1.9 to 2.69 | 64 | 72 |
| 29 to 30.9 | 77 | 40 | 52 | 36 | 2.7 to 3.49 | 16 | 22 |
| 31 to 32.9 | 114 | 43 | 56 | 52 | 3.5 to 4.29 | 2 | 3 |
| 33 to 34.9 | 265 | 94 | 103 | 77 | | | |
| 35 to 36.9 | 399 | 137 | 115 | 143 | | | |
| 37 to 38.9 | 89 | 41 | 31 | 35 | | | |
| 39 to 40.9 | 35 | 37 | 22 | 33 | | | |
| 41 to 42.9 | 9 | 7 | 2 | 9 | | | |
| 43 to 44.9 | 0 | 1 | 2 | 3 | | | |
| 45 to 46.9 | 0 | 0 | 2 | 1 | | | |

range of hosts and restriction of parasitism and also in its difference of effect on the host.

One of the main difficulties in determining the host range of the sorghum Sclerospora has been the failure to obtain artificial infection of the different hosts with the conidia produced on the living plants of sorghum or with the oospores placed on the germinating seed in the soil. After a number of unsuccessful attempts, however, sorghum plants were successfully inoculated in 1929 with the oospore material of the fungus, which was in a finely powdered condition. Experiments were then made, in which the seedlings of maize, teosinte, *Setaria italica, Pennisetum typhoideum,* and sorghum were exposed to infection with the oospores of the sorghum Sclerospora, and parallel inoculations were made with *Scl. graminicola.* Also, because its oospores resemble those of *Scl. sorghi,* similar inoculations were made with *Scl. noblei,* a species recently described by Weston (15), which occurs on the wild *Sorghum plumosum* Beauvois in the uplands of New South Wales, Australia, whence material was kindly furnished by its discoverer, Dr. R. J. Noble. The seed of each host was sown separately in sterilized soil in 6-in. pots and water was supplied by capillarity from below. Fine oospore material was placed on each seed in the soil. In all cases control pots were provided, in which no inoculum was dropped on the seeds.

TABLE 3.—*Summary of results obtained by inoculating different hosts with the oospores of Sclerospora sorghi, Scl. graminicola, and Scl. noblei*

| Host inoculated | Percentage of infected plants | | | |
|---|---|---|---|---|
| | Oospores of | | | |
| | *Scl. sorghi* | *Scl. graminicola* | | *Scl. noblei* |
| | | on *Pennisetum typhoideum* | on *Setaria italica* | |
| *Andropogon sorghum* | 32.2 | 0 | 0 | 0 |
| *Pennisetum typhoideum* | 0 | 21.4 | 0 | 0 |
| *Setaria italica* | 0 | 0 | 46 | 0 |
| *Euchlaena mexicana* | 16.0 | 0 | 14 | 0 |
| *Zea mays* (Golden Bantam) | 45.0 | 0 | 0 | 0 |
| *Zea mays* (Philippine variety) | 6.8 | 0 | 0 | 0 |
| *Zea mays* (Indian variety) | 44.0 | 0 | 0 | 0 |

The results are summarized in table 3 and show that the seedlings of sorghum, teosinte, and 3 varieties of maize were successfully inoculated

with the oospores of the sorghum Sclerospora. A more detailed account of the inoculation experiments with maize and teosinte has been given elsewhere (12). Negative results, however, were obtained with *Scl. noblei* and *Scl. graminicola*, the latter producing infection only on its hosts and on teosinte when the physiologic form on *Setaria italica* was used to inoculate it. In teosinte and maize the characteristic chlorosis of the leaves was noticed about a fortnight after inoculation, the symptoms in sorghum appearing as early as the 9th day after the seed was exposed to infection. When placed under a bell jar, the affected plants produced abundant conidial fructification within a week after the appearance of the first symptoms of chlorosis. These plants showed pale yellow blotching on the 2nd and 3rd leaf, and, as the seedlings developed, the other leaves also became infected. The conidia of *Scl. sorghi* thus produced germinated profusely by hyphae at 14–16° C., the minimum and maximum temperatures of germination being 10 and 30° C., respectively. The results on germination are tabulated in table 4.

TABLE 4.—*Relation of temperature to germination of conidia of Sclerospora sorghi*

| Temperature (° C.) | Percentage of germination in tap water |
|---|---|
| 6–8 | 0 |
| 8–10 | 0 |
| 10–12 | 50.0 |
| 12–14 | 88.0 |
| 14–16 | 90.0 |
| 16–18 | 85.0 |
| 18–20 | 65.0 |
| 20–22 | 50.0 |
| 22–24 | 52.0 |
| 24–26 | 50.0 |
| 30–31 | 0 |

The method used in germinating the oospores was essentially similar to that recently described by Hiura (7). The finely powdered spore material was dusted on blotting-paper supported partly on a wad of wet cotton wool placed at the bottom of a Petri dish. The lid of the Petri dish was lined inside with wet cotton wool, so that, on closing the lid, an atmosphere of high humidity was obtained within the Petri dish.

The dish was held at room temperature (23–25° C.) for about 48 hours. At the end of this period the spores had germinated by hyaline, unseptate germ tubes, which were usually branched (Fig. 1, C, 3 and 4); in some cases no branching was observed (Fig. 1, C, 2). The content of the oospore is hyaline, with a finely granular matrix, in which occur dense granular

bodies and 1 or 2 large masses of oil globules, which may be central or eccentric in position (Fig. 1, C, 1); but the germinating oospores show the granular bodies without the oily substance. In germination the wall of the oospore pushes out through the oogonial wall and grows into a germ tube, into which the contents of the oospore finally flow out. The germ tube varies in width from 2.5 to 8.3 μ, the average width being 4.4 μ.

DIAGNOSIS

The foregoing considerations seem to justify establishing this fungus as a distinct species with emended diagnosis as follows:—

*Sclerospora sorghi* (Kulk.) Weston and Uppal.

Syn. *Sclerospora graminicola* var. *Andropogonis sorghi* Kulkarni 1913 in Mem. Dept. Agr. India Bot. Ser., V. 5, No. 5, p. 268–273, pl. 6–7, 1 colored.

Conidiophores erect, spreading, comprising basal cell, main axis and more or less complex, usually dichotomously branched, expanded top. The basal cell knobbed or bulbous at the bottom, then of fairly uniform diameter (7–9 μ), for a length of approximately 100 to 150 μ until delimited usually by a complete septum, more rarely by a partial, ring-like thickening. The main axis expanding above to a diameter of 15 to 25 μ, and usually less than or equal to the basal cell in length, *i.e.*, extending about 80 to 150 μ, from the septum of the basal cell to the beginning of the branch system. The branch system comprising rapid successions of short, stout dichotomies usually involving primary, secondary, and tertiary branches terminating in tapering sterigmata usually about 13 μ long, the branches so arranged that the conidia borne on their tips lie approximately in a hemispherical plane.

Conidia suborbicular, varying from 15 to 28.9 μ x 15 to 26.9 μ, most frequently 21 to 24.9 μ x 19 to 22.9 μ, under natural conditions. Conidia hyaline, with a thin wall, continuous at the apex, unmodified, and quite without any papilla of dehiscence, hence germinating invariably by hyphae.

Oogonial stage resembling that of *Sclerospora graminicola* in general structural characteristics, such as the thick, irregularly polygonally-angled oogonial wall closely enveloping the single, hyaline, spherical oospore within, but differing slightly from *Scl. graminicola* on Pennisetum in effect on the host in that oospores, which develop chiefly within elongate reddish discolored areas in the mesophyll between the fibrovascular bundles, cause marked disintegration of the leaf tissue into tangled fibers. Oospores spherical, the majority being 31 to 36.9 μ in diameter, the mode being 35 to 36.9 μ, extremes ranging from 25 to 42.9 μ; wall a light shade of Mars Yellow (of Ridgway's "Color Standards"), most frequently from 1.1 to 2.7 μ thick, extremes ranging from 0.3 to 4.3 μ; content finely granular with

masses of oil globules, central or eccentric in position; germination by means of unseptate, usually branched, hyaline germ tube, averaging 4.4 μ in width, extremes ranging from 2.5 to 8.3 μ.

Occurring in both conidial and oogonial phase principally on sorghum (*Andropogon sorghum*) in the Bombay and Madras Presidencies, India, and rarely in its conidial phase on maize (*Zea mays*) near Poona, India, and also in Giza, Lower Egypt. Inoculated successfully on teosinte (*Euchlaena mexicana*). Very destructive to sorghum in Bombay and Madras Presidencies.

## SUMMARY

The history of this fungus, which was first recognized by Kulkarni, in 1913, as a variety, *Andropogonis sorghi*, of *Sclerospora graminicola* is reviewed.

Significant structural details of the conidial phase of this fungus on sorghum are compared with those of typical *Scl. graminicola* on Pennisetum and Setaria. The fungus on sorghum differs distinctively in its absolute lack of a dehiscence papilla in the apical wall of the conidia and their consequent germination by hyphae, and in the definite basal cell, extensive branch system, and consequent arrangement of the conidia in a hemispherical plane on the longer sterigmata of the conidiophores. On these features the fungus is separated from *Scl. graminicola* to specific rank as *Scl. sorghi*.

The oogonial phase resembles that of *Scl. graminicola* in general characteristics, but cross-inoculation experiments, using oospore material as inoculum, show that *Scl. sorghi* will not inoculate Setaria and Pennisetum, nor will *Scl. graminicola* from Pennisetum or Setaria inoculate sorghum, in spite of successful inoculations from each of these hosts to such remotely related Gramineae as *Euchlaena mexicana*. The results of these inoculations, which offer corroborative evidence for recognizing the fungus as a distinct species, are described and tabulated.

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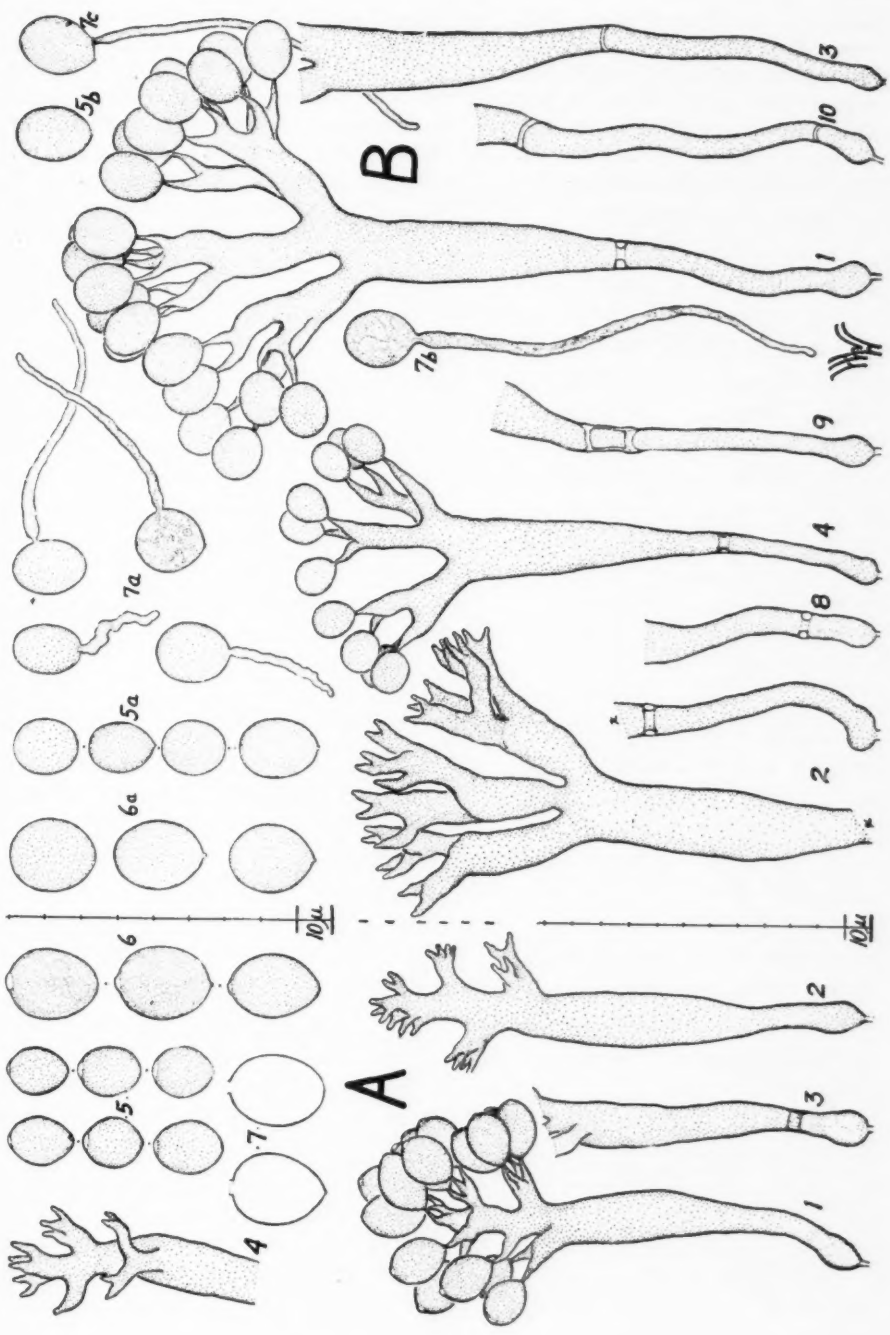
## EXPLANATION OF PLATE I

Comparison of *Sclerospora graminicola* (Sacc.) Schroet. A, 1–7, with *Sclerospora sorghi*, n. sp. B, 1–10. Drawings made with camera lucida from preserved material collected at Poona, India, during period of abundant conidiophore production. Magnifications apply to the present figures. Scales in microns.

A. *Sclerospora graminicola.* A, 1. Mature sporangiophore showing short, stocky habit, lack of basal cell, and main axis continuing into branch system with short laterals bearing ovate sporangia in groups. ×375. (*cf.* B, 1). A, 2. Similar sporangiophores after loss of sporangia showing typical habit, branch system, and sterigmata. ×375. (*cf.* B, 2). A, 3. Main axis of similar sporangiophore showing unusual occurrence of a true basal cell. ×375. (*cf.* B, 3). A, 4. Upper portion of a sporangiophore with branch system less typical yet showing continuance of main axis. ×375. A, 5. Mature sporangia of common shapes and sizes with characteristic papilla modification of apical wall through which zoospores emerge at germination. ×375. (*cf.* B, 5). A, 6. Similar sporangia enlarged, showing basal apiculus of attachment to sterigma, not to be confused with modified apical papilla. ×500. A, 7. Empty sporangial cases from which zoospores have emerged. ×500. (*cf.* germination by germ tubes in B, 6).

B. *Sclerospora sorghi.* B, 1. Mature conidiophore showing slender, isodiametric, relatively long basal cell, well developed "deliquescent" branch system, bearing true conidia in hemispherical arrangement. ×375. (*cf.* A, 1). B, 2. Similar conidiophore (after discharge of conidia) with characteristic spreading branch system and definite


A
B
10μ
10μ

basal cell. ×375. (*cf.* A, 2). B, 3. Main axis of conidiophore showing characteristic basal cell about equal in length to main axis above. ×375. (*cf.* A, 3). B, 4. Young depauperate conidiophore approximating in size those of *Scl. graminicola*, yet retaining characteristics of *Scl. sorghi*, *i.e.*, basal cell, expanding branch system, and hemispherically placed conidia. ×375. (*cf.* A, 1). B, 5. Representative mature conidia. Note lack of any apical papilla. ×375. (*cf.* A, 5). B, 6. Similar conidia enlarged. ×500. (*cf.* A, 6). B, 7. Conidia showing characteristic germination by hyphae. ×375. (*cf.* A, 7). B, 8. Nontypical short, broad, basal cell resembling those rarely occurring in *Scl. graminicola*. ×375. (*cf.* A, 3). B, 9 and 10. Basal cells showing aberrant septations occasionally seen. ×375.

# TWO NEW HOSTS OF THE DOWNY MILDEW OF SORGHUM IN BOMBAY[1]

B. N. Uppal and M. K. Desai

*Sclerospora graminicola* var. *Andropogonis-sorghi* Kulk. was described by Kulkarni in 1913 as the cause of the downy mildew of sorghum in the Bombay Presidency. He considered this fungus as a variety of *S. graminicola* (Sacc.) Schroet. showing distinct differences in its conidial stage and in its ability to infect *Pennisetum typhoideum* Rich. Until recently (4, 7), therefore, sorghum was the only host reported for this mildew. However, in 1929 some varieties of maize growing in the students' area on the College Farm, Poona, were badly affected by a downy mildew, and, since this disease had never been previously reported in the Bombay Presidency, its presence on maize was of special interest and led to the examination of the affected plants. It was surprising to find a very close resemblance between the conidia of the downy mildew on maize and those of the sorghum Sclerospora. It was, therefore, decided to make a detailed study of the downy mildew on maize and to determine whether it was possible to obtain artificial infection of this host with the oospores of *S. graminicola* var. *Andropogonis-sorghi.*

## COMPARATIVE STUDY OF THE SCLEROSPORA ON SORGHUM AND MAIZE

*Field symptoms.* As observed in the field, the symptoms and the general effects of the downy mildew on the individual plants of sorghum and maize were the same. The leaves of the affected plants were pale yellow and were covered with the conidial stage of the fungus. These symptoms were noticeable when the plants were about 5 weeks old. At a later stage sorghum plants showed the characteristic shredding of the affected leaves, but no such effects were produced in maize, which continued to support the conidial fructification on the pallid areas of the leaves. The fungus did not produce oospores in the leaf tissues of the maize.

*Infection experiments.* Since it has been possible to infect sorghum with the oospores of *Sclerospora graminicola* var. *Andropogonis-sorghi* (6), experiments were made to determine whether the seedlings of maize and teosinte (*Euchlaena mexicana* Schrad.) could be successfully inoculated with this variety. Teosinte was included in these studies, since Butler has reported this as a host of *S. graminicola* on *Pennisetum typhoideum,* which has, however, recently been shown by the writers (8) not to infect teosinte under controlled conditions. Butler (2, pp. 193 and 223) has already expressed a doubt as to the identity of the downy mildew on teosinte

[1] Published with the approval of the Director of Agriculture, Bombay Presidency.

TABLE 1.—*Results obtained from exposing 2 varieties of maize to infection by oospores of Sclerospora graminicola var. Andropogonis-sorghi, 1929*

| Plants exposed to infection | Oospores from | Number of plants exposed | Percentage of plants infected |
|---|---|---|---|
| *Zea mays* (local) | *Andropogon sorghum* | 6 | 50.0 |
| Control[a] | Nil | 6 | 0.0 |
| *Z. mays* (Golden Bantam) | *A. sorghum* | 7 | 57.0 |
| Control[a] | Nil | 7 | 0.0 |

[a] Control plants remained healthy in all cases.

but has suggested that *S. mydis* (Rac.) Butl. might be the species concerned because of the absence of the oosporic stage on this host. However, since this species has not been recorded in Bombay, the identity of the downy mildew on teosinte first seen by Butler (1), in 1905, on the Poona Government Farm, became very doubtful.

*1929 experiment.* On August 15, 1929, a variety of maize—sweet corn (Golden Bantam)—and local maize were sown in sterilized soil in 6-inch pots. The seed bed was watered before the seed was sown. In each pot small holes, about 2 to 3 in. deep, were made in the seed bed, and oospore material of the sorghum Sclerospora was dropped at the bottom of each hole. A seed was sown in each hole, and the oospore material was again dropped on each seed. The soil was restored on the seed. To prevent the oospores from being washed down, the pots were allowed to stand in China dishes full of water so that water was supplied to the germinating seeds by capillarity, thus ensuring against the least disturbance to the physical contact of the oospores with the seed. In each case control pots were provided in which no inoculum was dropped.

A reference to table 1 will show that the seedlings of sweet corn (Golden Bantam) and local maize were successfully inoculated with the oospores of the downy mildew of sorghum. In Golden Bantam the characteristic chlorosis of leaves was noticed 14 days after the seed was exposed to infection; but, in the seedlings of local maize, these symptoms did not appear until the 19th day after inoculation. When placed under a bell jar, the affected plants produced conidial fructification within a week of the appearance of the first symptoms of the downy mildew. These plants showed pale yellow blotching on the 2nd or 3rd leaf, and, as the seedlings developed, the other leaves also became infected. Sporulation was obtained also on

leaves placed in Petri dishes lined with wet blotting paper and exposed to tap water at 17–18.5° C. for about 9 hours. There was, however, profuse sporulation when these leaves were left overnight in Petri dishes at room temperature (20–22° C.). In all tests conidia germinated by the protrusion of 1 or 2 germ tubes.

*1930 experiment.* Besides the varieties of maize tested in 1929, teosinte and Philippine maize were included in the trials made in 1930. The method of inoculation was essentially the same as described above. In addition to the 6-inch pots, wooden flats were filled with sterilized soil and the seed beds were thoroughly wet down before the seeds were sown. In each seed bed furrows were opened, and the seed was moistened, rolled in fine oospore material and sown in the furrows. Oospores were again dusted on the seeds through a fine muslin cloth. The soil was finally restored to the furrows. In all cases water was supplied by capillarity. Control plants were provided for each series. The seeds germinated well in all cases.

TABLE 2.—*Results obtained from exposing varieties of maize and teosinte to infection by oospores of Sclerospora graminicola var. Andropogonis-sorghi, 1930*

| Plants[a] exposed to infection | Oospores from | Number of plants exposed | Percentage of plants infected |
|---|---|---|---|
| *Zea mays* (local) | *Andropogon sorghum* | 54 | 38.8 |
| " " (Philippine) | " " | 29 | 6.8 |
| " " (Golden Bantam) | " " | 29 | 34.4 |
| *Euchlaena mexicana* | " " | 50 | 16.0 |

[a] Control plants remained healthy in all cases.

It will be seen from table 2 that all the varieties of maize and teosinte were infected with the oospores of the downy mildew of sorghum, although the percentage of infection in the Philippine maize was low. In a few cases the symptoms were first noticed as early as the 14th day after inoculation, but in some others they did not appear before the 25th day or even before the lapse of a month. Sporulation was readily obtained on affected plants placed under a bell jar or on leaves in Petri dishes subjected to tap water. The condia germinated by the formation of 1 or 2 germ tubes.

## MORPHOLOGICAL CHARACTERS

After it had been ascertained that the downy mildew of sorghum can infect maize under controlled conditions, it was decided to make a comparative morphological study of the conidial phase of the mildew as it

occurs naturally on maize and sorghum in Bombay. The material for study was obtained from plants growing in the field during the time of optimum conidiophore production at night between 3 and 4 a. m. The leaves of naturally infected plants were wiped clean of their previous sporulation with wet cotton wool, in the evening, and a rich crop of conidia was obtained in the following night a few hours after midnight. The conidia were mounted in water and the measurements were invariably made before sunrise.

*Conidiophores.* In general appearance the conidiophores of the Sclerospora on sorghum were exactly similar to those of the downy mildew on maize. (Fig. 1, 1, 2 and 1a, 2a). Each was provided with a basal cell, which had usually 1 septum separating it from the rest of the main axis. (Fig. 1, 1 and 1a, 2a). In the Sclerospora on sorghum sometimes 2 septa were seen in the basal cell. (Fig. 1, 2 and 3). The average length of the

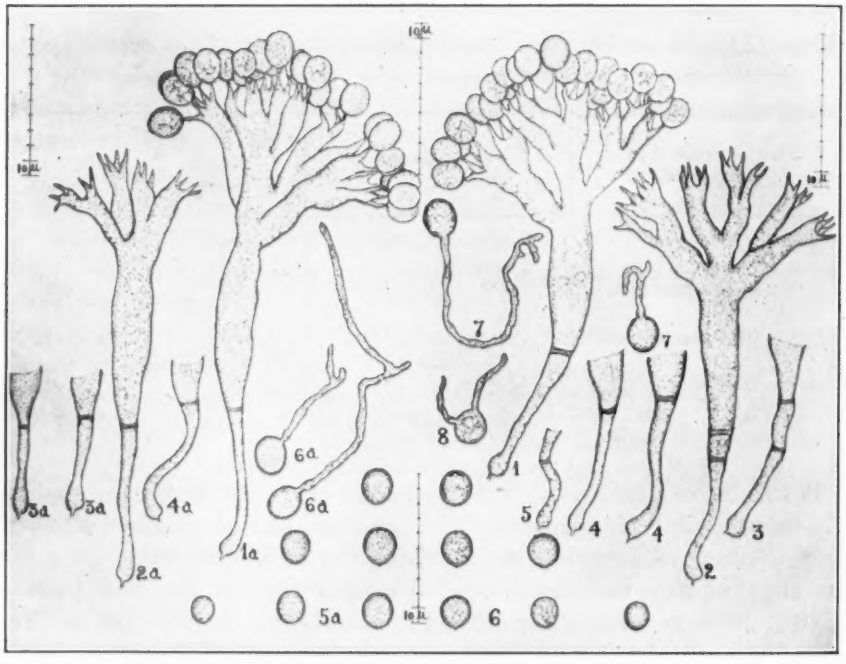



FIG. 1. Comparison of *Sclerospora graminicola* var. *Andropogonis-sorghi* on *Andropogon sorghum* and *Zea mays:* On *A. sorghum*—1, a well-developed conidiophore with a basal cell; 2, 3, basal cells with 2 septa; 4, basal cells of conidiophores of length normally encountered; 5, a part of a conidiophore showing a basal cell much shorter than normally encountered; 6, conidia showing range of shapes and sizes; 7, 8, germinating conidia with 1 or 2 germ tubes. On *Z. mays*—1a, same as in 1; 2a, a sparsely branched conidiophore with conidia fallen off; 3a, 4a, same as in 4; 5a, same as in 6; 6a, germinating conidia with 1 germ tube.

conidiophores of *Sclerospora graminicola* var. *Andropogonis-sorghi* was about 200 μ.

*Conidia.* In shape and size the conidia of the Sclerospora on maize agreed very closely with those of *S. graminicola* var. *Andropogonis-sorghi.* (Fig. 1, 6 and 5a). They are suborbicular and lack an apical papilla of dehiscence. They, therefore, invariably germinate by the formation of 1 or 2 germ tubes. (Fig. 1, 7, 8, and 6a).

In order to obtain a correct impression of the conidial dimensions, 400 conidia, each, from maize and sorghum were measured and arranged in a series of classes. It will be seen from table 3 that the measurements of the

TABLE 3.—*Summarized measurements of conidia of the Sclerosporas on sorghum and maize*

| Classes in μ | Length | | Classes in μ | Width | |
|---|---|---|---|---|---|
| | Number of conidia in 400 | | | Number of conidia in 400 | |
| | Sorghum | *Zea mays* | | Sorghum | *Zea mays* |
| 15 to 16.9 | 6 | 7 | 15 to 16.9 | 20 | 16 |
| 17 to 18.9 | 9 | 8 | 17 to 18.9 | 29 | 27 |
| 19 to 20.9 | 98 | 108 | 19 to 20.9 | 180 | 180 |
| 21 to 22.9 | 112 | 115 | 21 to 22.9 | 113 | 114 |
| 23 to 24.9 | 132 | 114 | 23 to 24.9 | 53 | 50 |
| 25 to 26.9 | 40 | 45 | 25 to 26.9 | 5 | 13 |
| 27 to 28.9 | 3 | 3 | 27 to 28.9 | 0 | 0 |

length and width of conidia produced on sorghum are in complete agreement with similar measurements of conidia taken from maize.

The biometrical constants of the conidial dimensions are given in table 4. An examination of the data will show that the standard deviations for length and width in the case of the Sclerosporas on sorghum and maize are almost the same. For example, the standard deviation for the length of conidia is $2.21 \pm 0.052$ in the case of the Sclerospora on sorghum and $2.26 \pm 0.053$ in the case of the fungus on maize, and for width the deviations are $2.02 \pm 0.048$ and $2.07 \pm 0.049$, respectively. Likewise, the coefficients of variability, both for length and width, also agree very closely. Also, the data presented in table 5 indicate that the differences in the means of length and width of the conidia of both the Sclerosporas are very insig-

TABLE 4.—*Biometric constants for length and width of the Sclerosporas on sorghum and maize*

| Sclerospora on | Mean | Mode | Standard deviation | Coefficient of variability |
|---|---|---|---|---|
| **Length** | | | | |
| *Andropogon sorghum* | 22.43 ± 0.072 | 23.9 | 2.21 ± 0.052 | 9.8 ± 0.23 |
| *Zea mays* | 22.34 ± 0.07 | 21.9 | 2.26 ± 0.053 | 10.1 ± 0.24 |
| **Width** | | | | |
| *Andropogon sorghum* | 20.82 ± 0.068 | 19.9 | 2.02 ± 0.048 | 9.7 ± 0.23 |
| *Zea mays* | 20.97 ± 0.069 | 19.9 | 2.07 ± 0.049 | 9.8 ± 0.23 |

nificant; that is, the odds against the occurrence of the differences as great as or greater than 0.941 in the case of length and 1.535 in the case of width are about 1 : 1 and 2.21 : 1, respectively. In other words, the differences for length and width of the conidia of the Sclerosporas on sorghum and maize have no statistical significance and may be due to random fluctuation, thus indicating that the 2 fungi are identical.

## DISCUSSION

The experimental data above presented clearly indicate that the downy mildew on maize, which is found occurring naturally in the Bombay Presidency in certain seasons, is identical with *Sclerospora graminicola* var. *Andropogonis-sorghi* both in its effects on the host and in the morphological characters. This fungus must now be added to the list of 7 species of Sclerospora already known to attack maize.

The occurrence of the sorghum Sclerospora on maize is of special interest, since the development of the sexual stage, which is a prominent char-

TABLE 5.—*Differences in means of the Sclerospora on sorghum and maize relative to the probable errors*

| Difference in means | | Difference in means divided by probable error of difference | | Odds (approximately) | |
|---|---|---|---|---|---|
| Length | Width | Length | Width | Length | Width |
| 0.095 ± 0.10 | 0.15 ± 0.097 | 0.941 | 1.535 | 1.00 : 1 | 2.21 : 1 |

acter of this fungus on sorghum, is completely suppressed in maize. This phenomenon, however, is not peculiar to the sorghum Sclerospora, since *Sclerospora graminicola,* occurring on *Setaria viridis* (L.) Beauv. and others, has also been shown not to produce oospores in maize (5). The question arises as to how a species of Sclerospora that normally produces the oogonial stage can be so altered by its passage from its host to maize that its sexual stage is suppressed in the latter host. Clinton (3) has reported a similar relationship in *Phytophthora infestans* (Mont.) de Bary, which can be induced to produce oospores more readily on oatmeal agar than on other media. However, the relation of food materials to the development of the oogonial stage in the species of Sclerospora cannot be determined until it is possible to grow these fungi in artificial culture.

In 1905 Butler first saw a downy mildew on teosinte, *Euchlaena mexicana,* on the Government Farm, Poona. Since *Sclerospora graminicola* was commonly found on *Pennisetum typhoideum* growing in the vicinity, Butler was led to believe this mildew occurred on teosinte in Bombay, a statement he later modified, as this mildew was not seen to produce the oogonial stage in the latter host. It is very likely that Butler (1) came to this conclusion because he could not find the conidial stage of the Sclerospora on sorghum, which he then considered to agree with the species on Pennisetum. It, therefore, seems very reasonable to believe that the mildew on teosinte, seen by Butler in 1905, was, in fact, the form on sorghum, for it has recently been shown by the writers (8) that *S. graminicola* is split into specialized races and that the form on *P. typhoideum* fails to infect teosinte. Moreover, this conclusion is justified in view of the fact that *S. graminicola* var. *Andropogonis-sorghi* is found very commonly on sorghum in Bombay, while *S. maydis* (Rac.) Butl., later considered by Butler (2, pp. 193 and 223) as the cause of the downy mildew of teosinte, has never been recorded in this Presidency.

## SUMMARY

1. The downy mildew of maize occurring naturally on this host in Bombay has been found to be caused by *Sclerospora graminicola* var. *Andropogonis-sorghi.* In the conidial stage it is not possible to distinguish between the mildews on sorghum and maize. The sexual stage is prominent on sorghum, resulting in the characteristic shredding of leaves, but it is completely suppressed in maize.

2. Maize and teosinte (*Euchlaena mexicana*) have been infected with the oospores of *S. graminicola* var. *Andropogonis-sorghi* under controlled conditions.

3. A comparative morphological study of the conidial phase of the mildew as it occurs naturally on maize and sorghum in Bombay has been made.

In general appearance the conidiophores are alike, and each is provided with a basal cell. The biometrical measurements of the conidia of the mildews on maize and sorghum have shown that the differences in length and width of conidia are not statistically significant, thus indicating that the 2 fungi are identical.

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# CHEMICAL TREATMENT OF PECAN ROSETTE[1]

A. O. Alben, J. R. Cole, and R. D. Lewis

## INTRODUCTION

Pecan rosette, a disease apparently of nonparasitic origin, is present in every state and on almost every soil type where pecans are grown commercially. In some of the Southeastern States, hundreds of acres of pecan orchards made unprofitable by this disease have been abandoned and relatively few of the commercial plantings in the whole pecan territory are entirely free from it. In the Southwestern States where the commercial plantings are more recent, and more especially on the basic soils, as high as 95 per cent of the trees are rosetting in some localities. This is true of nursery trees as well as of planted orchards. In spite of the fact that growers are using more care in selecting orchard sites as well as taking better care of their orchards, rosette is now a very serious problem to nurserymen and orchardists alike.

Yellow mottling, or chlorosis, and crinkling of the leaves are characteristic of rosette. In orchards the tops of trees seem to be first affected, with a gradual spread to the lower branches. The disease progresses from a mild chlorosis of the leaves to a dwarfing of the leaves, shortening of the internodes, and, finally, to death of twigs and branches. While few, if any, trees die from rosette, severely rosetted trees are generally nonproductive and become so weakened that they die from attacks or borers or from other causes.

Rosette occurs under varying conditions on both residual and alluvial soils. Eroded areas, a tight subsoil condition, light soil textures, and deficiencies in organic matter seem to favor the occurrence of rosette on the residual soils. A high water table and soils of light texture favor rosette on the alluvial bottoms. Well-drained, fertile, medium to heavy soils types are generally free from rosette except under special conditions. On the alluvial soils of Louisiana, rosette occurs regardless of soil type when trees are growing near old building sites, near negro cabins, in extremely fertile garden soils, in barn lots, and near pigpens.

A varietal resistance or susceptibility to rosette seems to exist. The Stuart is the most susceptible, followed closely by the Frotscher and Van Deman varieties, while the Moneymaker seems to be highly resistant. The writers have never seen native pecan trees rosetting in their natural state in the woods. Native pecans left standing and brought under cultivation when

[1] Cooperative investigations between the Bureau of Chemistry and Soils and the Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

the woods were cleared away have rosetted severely. Certain culture practices seem to favor the development of rosette, while others seem to hold it in abeyance or affect a cure.

## REVIEW OF LITERATURE

Orton and Rand (4), in 1914, reported that no parasitic organism was associated with this disease. Their investigations indicated that the trouble lay in the soil, but whether the cause was physical, chemical, or biological was not indicated.

In 1919, McMurran (3), who worked on rosette from 1914 to 1920, reported that from "10 to 20 per cent of the pecans planted in orchards in the southeastern United States have shown this trouble in a more or less marked degree." After making a survey of pecan orchards from Florida to eastern Louisiana, McMurran also reported in the same publication that "while the disease was seen to be present on a wide range of soil types and under various conditions of cultivation and fertilization, one fact seemed to stand out prominently. A very large proportion of the diseased trees, probably 90 per cent or more, was found on the hilltops and slopes. Occasional cases or groups of cases were on bottom lands, but all of these that have been examined have been found to be growing in deep sand or else in a clay or sand clay underlain at 2 to 3 feet by sand. It was observed also that wherever the conditions were such as to produce any quantity of rosette, the weeds or crops growing in the tree rows had a stunted, yellow, unthrifty appearance as compared with those growing among healthy trees, . . . On the other hand, trees planted in low places in which humus and fertility had accumulated from year to year, were uniformly vigorous, thrifty, and free from disease."

McMurran further reported that "While apparently contradictory cases occasionally are seen, 90 per cent or more are surrounded by conditions that plainly indicate a deficiency of humus, plant-food material, and moisture. Either where the soil or topography is such that there is a deficiency of this combination, or where the tree's condition is such as to interfere with its obtaining an adequate supply of plant-food material, the result appears to be the same, *i.e.*, a yellowing of the foliage, a shortening of the internodes, a pushing out of dormant buds, and in many cases a dying back of the twigs and branches."

In 1922, Rand (5) concluded that rosette is "more in agreement with the infectious type of chloroses, including the yellows and mosaic groups, than with those chloroses known to be caused directly by soil or climatic conditions."

Skinner and Demaree (6), in their experimental work with rosette in southern Georgia, were able to bring badly rosetted trees that were growing

on soils low in organic matter back to normal by growing and turning under two green manure crops each year. Chemical fertilizers as used in the experiment had no influence in increasing or decreasing rosette.

### EXPERIMENTS IN CONTROL

As shown by the literature as well as observation by the writers, the evidence very strongly favors the view that rosette is of non-parasitic origin

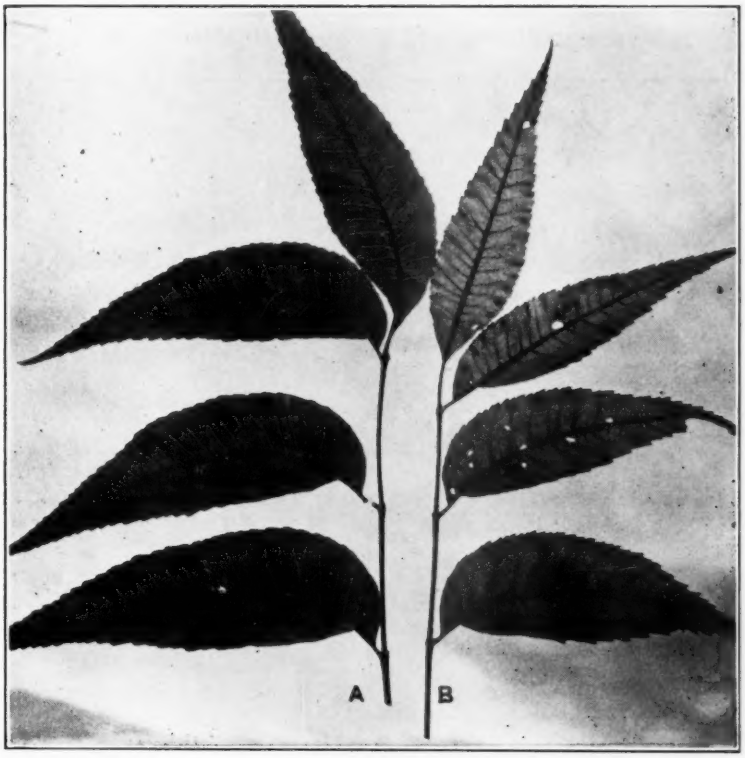


FIG. 1. A. Part of a pecan leaf that, 30 days before, had been dipped in a 1 per cent ferric sulphate solution. B. Part of a nontreated leaf from the same terminal as A. A is normal but B is almost devoid of chlorophyll.

and that its development depends on the presence of some soil factor or factors at present not definitely known. The disease does not seem to spread from badly rosetted trees to healthy ones, though their branches may be interlocking. Most of the trees in a locality seem to rosette simultaneously. Rosetted leaves and their extracted juices, when placed on healthy leaves, have failed to produce rosette.

Because of the increasing importance of rosette in the Southwestern States, the writers outlined a series of experiments in 1930 and expanded

them in 1931, with the hope of determining the cause of rosette. In these experiments, three different methods were used: first, culture practices; second, applications of chemicals to the soils; and third, application of chemicals in solution to the leaves.

The culture practices have consisted in (1) clean cultivation; (2) the growing of a cover crop that was allowed to stand. No results have been obtained, since the experiment has run but one season.

Calcium sulphate, manganese sulphate, magnesium sulphate, and potassium chloride were applied to both hill and bottom-land soils in April, 1930

FIG. 2. Part of a pecan leaf. One leaflet, A, received an application of ferric sulphate solution (0.8%). At the time the photograph was taken, 12 days after treatment, the treated leaflet was almost normal and increased in size over its companion nontreated leaflet.

and 1931, and plowed under; in addition, ferrous and ferric sulphate were placed in trenches in August, 1931, on bottom-land soils. None of these treatments has shown any apparent beneficial effects up to the present time.

Calcium sulphate, manganese sulphate, magnesium sulphate, potassium bromide, potassium sulphate, aluminum sulphate, boric acid, hydrochloric acid, and sulphuric acid, in varying strengths, were sprayed on trees May 26, 1931, without any apparent beneficial results. Ferric sulphate and

ferric chloride, sprayed on Schley and Stuart trees, August 1, 1931, either improved the condition of rosetted leaves or brought them back to normal, depending on their stage of growth.

Rosetted leaves were treated with both ferric sulphate and ferric chloride in strengths varying from 0.1 to 1 per cent. Improvement was noted in the more dilute applications ranging from 0.1 to 0.5 per cent. Complete recovery was effected with strengths ranging from 0.6 to 1 per cent. Slight

FIG. 3. A pecan leaf. Ferric sulphate solution (0.8%) was applied to the two leaflets indicated by arrows. The upper treated leaflet has almost completely recovered and the lower one shows improvement. Photographed 12 days after treatment.

burning with subsequent recovery resulted from the use of 0.8 to 1 per cent dilution. An effort was made to prevent the burning effect of the stronger solutions by neutralizing their acidity with lime. The results of this experiment were an increase in burning and no improvement of the rosette condition. Figures 1, 2, 3, and 4 show some of the results obtained by applying solutions of iron salts to pecan leaves.

## DISCUSSION AND CONCLUSIONS

The evidence of a single season's treatments must, of course, be recognized as inconclusive, partly because spraying treatments were carried out in the latter part of the growing season rather than throughout the entire growing period and partly because of the possibility of the mid- or late-season treatments' masking chlorotic symptoms that might become apparent in the following season. Admitting the possibility of contradictory results in large-scale experiments and on other soil types and other varieties, it appears that the applications of solutions of iron salts to rosetted leaves of Stuart and Schley varieties cured or, at least, lessened the severity of rosette on trees growing in residual and in alluvial soils.

FIG. 4. Left, a rosetted pecan terminal that had been dipped in a 1 per cent ferric chloride solution with a normal leaf that developed after treatment; right, a near-by nontreated rosetted terminal. Photographed 45 days after treatment.

These findings would seem to indicate that pecan rosette is related to iron chlorosis. Iron chlorosis of other plants due to a high lime content of the soil has been known for years. As early as 1843, E. Gris (2) of France first found that iron sulphate applied to chlorotic plants through roots or leaves caused them to become green. Since that time, treatment of chlorosis with iron salts has been more or less common in Europe. In recent years (1) treatment of pears, peaches, apples, citrus, and other plants in the United States for lime-induced chlorosis has been much practiced. Pecan rosette, however, occurs on both acid and basic soils. Many facts have yet

to be brought to light and correlated before the cause of rosette can be satisfactorily explained. With work and experiments now in progress, the writers hope to throw more light on the cause of rosette and develop methods for curing or preventing this disease.

SUMMARY

Pecan rosette is apparently a nonparasitic disease of wide-spread distribution occurring on both residual and alluvial soils. A varietal resistance or susceptibility to rosette seems to exist. The Stuart is the most susceptible, while the Moneymaker seems to be highly resistant.

The writers were able to improve old rosetted leaves and bring the young ones back to normal by dipping or spraying them with a solution of ferric sulphate or ferric chloride, ranging in strength from 0.6 to 1 per cent. These findings would seem to indicate that pecan rosette is a condition of iron chlorosis.

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# EFFICIENCY OF OILED WRAPS IN THE COMMERCIAL CONTROL OF APPLE SCALD

NEIL E. STEVENS AND NELLIE W. NANCE

That the commercial control of plant diseases frequently fails to approximate the degree of control obtained in experiments is a common observation. Relatively little information is available as to the actual results obtained in commercial practice, due largely to the difficulty of accurately measuring such results. The intensive surveys of 1929 and 1930 demonstrated that to an extent not previously recognized the commercial control of stinking smut of wheat often falls below experimental control.[1]

The present paper is an attempt to evaluate the actual commercial efficiency of the oiled wraps in the control of apple scald, as developed by Brooks and his associates.[2] The information contained in the inspection certificates of the Food Products Inspection Service of the Bureau of Agricultural Economics, United States Department of Agriculture, forms the basis of this study. The summary is confined to the boxed apples of Washington State, since in this region the control measure was most promptly and generally adopted.

## THE "OILED-WRAPS" METHOD OF CONTROL AND ITS ADOPTION

As is well known, practically all of the apples shipped in boxes from the Pacific Northwest of the United States have long been packed in individual paper wraps. The method for the control of apple scald here referred to is the use of paper impregnated with mineral oil instead of the untreated paper formerly used.

Several influences combined to give this control method prompt and wide application. The disease is easily distinguished, and had long been recognized as serious. The control method involved only a slight change in the commercial handling of boxed apples. The discoverers had the confidence of the industry and were actively engaged in field work in large producing centers. The industry itself is highly organized in the regions from which most of the boxed apples are shipped.

A statement of the rate of adoption is found in Brooks'[3] paper of August, 1924. In the 1922 crop, about 600,000 boxes of apples in the North-

[1] Haskell, R. J., R. C. Rose, W. E. Brentzel, E. A. Walker, and Waldo Kidder. Why so much smut in spring wheat? U. S. Dept. Agr., Bur. Plant Indus., Plant Dis. Rptr. Sup. 77: 97–138. 1930. (Mimeographed).

[2] Brooks, Charles, J. S. Cooley and D. F. Fisher. Apple scald and its control. U. S. Dept. Agr. Farmers' Bul. 1380. 1923.

[3] Brooks, Charles. Apple scald. Off. Proc. Inter. Apple Shippers' Assoc. 29: 154–158. 1924.

west went into storage in oiled wraps. In the summer of 1923, about 15,000,000 bushels were so treated. By 1924, the use of oiled wraps was almost universal in Washington, and had been adopted to some extent throughout the apple-growing regions of the country. For the barrelled fruit the method was modified by the use of shredded paper, scattered among the apples.

### COMMERCIAL RESULTS IN THE CONTROL OF APPLE SCALD IN THE STATE OF WASHINGTON

As indicated in the preceding paragraph, a small proportion of the Washington apple crop was packed in oiled wraps in 1922, a much larger amount in 1923, and by 1924 the method had been generally adopted. Since that time it has continued in popularity and has become a part of regular commercial practice in that region.

The information on scald in apples during this interesting period, obtainable from the inspection certificates of the Food Products Inspection Service, is summarized in table 1. Calculated on the basis of all cars

TABLE 1.—*Scald and blue mold in boxed apples from Washington. Calculated on the basis of all cars inspected*

| Crop year | Total cars | Percentage of cars showing scald | Average percentage of scald | Average percentage of blue mold |
|---|---|---|---|---|
| 1922 | 3,515 | 16.4 | 2.8 | 1.8 |
| 1923 | 2,781 | 5.3 | 0.7 | 0.9 |
| 1924 | 1,713 | 1.8 | 0.3 | 1.7 |
| 1925 | 1,973 | 2.2 | 0.2 | 1.2 |
| 1926 | 1,903 | 1.9 | 0.2 | 1.5 |
| 1927 | 1,825 | 3.4 | 0.3 | 1.8 |
| 1928 | 1,694 | 3.7 | 0.4 | 1.6 |
| 1929 | 2,092 | 1.9 | 0.2 | 2.0 |
| 1930 | 3,811 | 2.0 | 0.2 | 0.6 |

inspected, the percentage of scald in 1922 was 2.8; in 1923 it fell to 0.7; and during the succeeding years it has maintained an average of less than 0.3 per cent, or approximately $\frac{1}{10}$ the amount for 1922. These percentages are striking, but they do not show the actual effect of the control measure, since the number of cars showing scald had decreased so markedly as to reduce the total number of cars inspected.

The same source of error occurs in the calculation of the percentage of cars that show scald, although this gives a more accurate picture. As indicated in column 3 of table 1 and in the graph, figure 1, the percentage

TABLE 2.—*Scald in boxed apples from Washington*

| Crop year | Total cars inspected that showed disease of any kind | Average percentage of scald calculated on the basis of all cars showing diseases of any kind | Average percentage of scald in cars that showed this disease |
|---|---|---|---|
| 1917–1920 | 3,095 | 5.2 | ..... |
| 1922 | 2,002 | 4.9 | 17.0 |
| 1923 | 852 | 2.4 | 14.0 |
| 1924 | 693 | 0.7 | 14.7 |
| 1925 | 636 | 0.7 | 10.5 |
| 1926 | 757 | 0.6 | 11.5 |
| 1927 | 842 | 0.6 | 7.4 |
| 1928 | 743 | 0.8 | 9.5 |
| 1929 | 874 | 0.4 | 8.8 |
| 1930 | 997 | 0.8 | 10.5 |

of inspected cars showing scald was 16.4 in 1922, fell to 5.3 in 1923, and in the succeeding years has averaged approximately 2.5, which is somewhat less than $\frac{1}{6}$ of the amount for 1922.

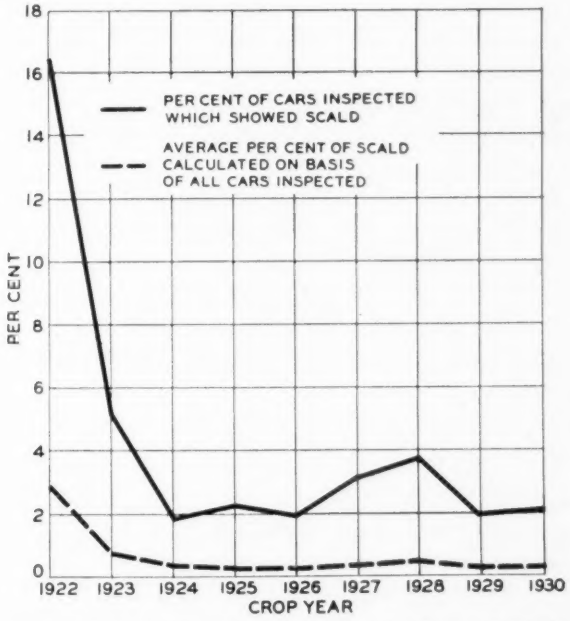


FIG. 1. Amount of scald in boxed apples from the State of Washington as shown by the inspection certificates of the Food Products Inspection Service, Bureau of Agricultural Economics, U. S. Department of Agriculture.

That the crop of 1922 was not characterized by an abnormally large amount of scald is shown in table 2, in which the average amount of scald for the period 1917 to 1920, as calculated by Rose,[4] is compared with that of the succeeding years. For the purpose of direct comparison with Rose's figures, the percentage is here calculated on the basis of cars that showed disease of any kind. Column 3, table 2, shows that the amount of scald for 1922 was slightly below the average for the years 1917 to 1920. 1923 shows a reduction to somewhat less than ½ of the previous amount, and the succeeding years have continued to show a very small amount of this disease.

In the last column of table 2 is indicated the amount of scald in the cars that showed this disease. Some decrease after the introduction of the treatment is evident, but, as would be expected, the greatest reduction is shown in the number of cars in which scald developed.

### LOSSES FROM BLUE MOLD COMPARED WITH SCALD

In order to eliminate the possibility that general conditions had changed so markedly as to affect the general quality of the shipped apples, wholly independent of this special treatment, a computation was made of the losses from blue mold during the same period. Although there is, of course, some variation in the losses from blue mold, calculated on the basis of all cars inspected, there is no evidence of any change similar to that shown for scald. The losses were, in general, somewhat less than those from scald before the control method was developed but very much larger than those from scald under the later conditions.

It is not to be supposed that the figures taken from the inspection sheets and here summarized give a complete picture of the amount of scald in Washington apples throughout the period under review, but these inspections based on over 20,000 cars, made by trained men in central markets throughout the country, must have some significance. It is probably not too much to claim that they furnish the best obtainable indication of the commercial conditions.

Perhaps the most significant fact brought out by this summary is that the number of inspections requested fell in 1924, after oiled wraps became generally used, to 50 per cent of the number requested in 1922. Although the inspection service continued in popularity and in the confidence of the trade, the number of inspections requested on Washington apples did not for several years approximate the number requested in 1923 and even as late as 1929 was less than 60 per cent of that in 1922.

[4] Rose, D. H. Diseases of apples on the market. U. S. Dept. Agr. Bul. 1253. 1924.

### COMMERCIAL IMPORTANCE

It is probably unnecessary to call attention to the fact that the figures presented in this summary, while striking, are by no means an indication of the commercial importance of the control method. An attempt to evaluate the actual significance of this single change in commercial practice would necessitate the consideration of many interesting but highly speculative factors, among which would be an attempt to determine the relative value and salability of lots of apples having less than 1 per cent scald and those having over 5 per cent, the effect on the general apple and fruit market of the presence of numerous cars of badly scalded fruit, and the relation of other diseases to the presence of scald.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

# SOME DISEASES OF WILD POTATOES IN MEXICO

DONALD REDDICK

The discovery that *Solanum demissum* Lindl. is immune from the blight caused by *Phytophthora infestans* has directed attention to Mexico as a possible source of breeding stock for a permanent improvement of the potato through the production of disease-resistant sorts. When Humboldt[1] explored in Mexico at the beginning of the last century he announced that he found no potatoes in the Republic. His statement has been quoted repeatedly until very recent times. In the meantime, however, about 30 species of tuberous Solani have been described from Mexico many of them endemic to that country. There is very little evidence that Mexican species have entered into the production of the cultivated plant known as *S. tuberosum*. The possibility that *S. demissum* blood may be expressing itself at the present time in certain German varieties through the hybrids made by Klotzsch has been suggested elsewhere.[2] *Solanum verrucosum,* another Mexican endemophyte, was grown for a short time in Switzerland but was discarded because of its susceptibility to blight, apparently without making any impression on the cultivated sorts.

There is little evidence that potatoes ever have played an important rôle in Mexico. The important food sources seem to have been maize (Zea) and beans (Phaseolus). These crops can now be found at all levels, in Mexico, from the sea to an elevation of 10,000 ft. Certainly there is nothing in Mexican archeology comparable to the unmistakable pottery tubers of the Peruvian indigenes. The Spaniards in Mexico unquestionably uprooted many wild potatoes when they constructed walls for their churches and monasteries. Wild potatoes of at least 2 different species can be found now about the monastery at El Desierto, a structure erected fairly early in the 16th century. These plants occur in the ruins of the wall, the church itself, and far up the mountains in deep humic soil under coniferous trees. But the conquistadores were interested in other kinds of nuggets; and in saving souls. There seems to be no evidence whatever that these nuggets

[1] Humboldt, Al. de. Essai politique sur le royaume de la Nouvelle-Espagne. 5 v. F. Schoell, Paris. 1811. Citation in v. 3, pp. 107–108.

In the English translation by John Black (Political essay on the kingdom of New Spain. 2 v. I. Riley, New York. 1811) the statement will be found in v. 2, p. 342.

[2] Reddick, Donald. Frost-tolerant and blight-resistant potatoes. Phytopath. 20: 987–991. 1930.

of starch were taken back to Spain and certainly nothing in present-day varieties to suggest such a thing.

In October, November, and December, 1930, an effort was made by the Division of Foreign Plant Introduction, U. S. Department of Agriculture, to assemble living material of as many as possible of the Mexican species of tuber-bearing Solani. No attempt was made to secure *Solanum fendleri* or *jamesii* from Mexico but, aside from these species, all of the places in Mexico from which wild potatoes have been reported were visited and, in all, perhaps a dozen species were secured. Wild plants were seen in a variety of conditions and all were scrutinized for the presence of disease.

### RUST

Rust was found repeatedly on *Solanum demissum* at El Desierto des Leones, now a national preserve about 40 km. from Mexico City, but was never found elsewhere. When first encountered in October, 1930, the spots were just forming and it appeared that aecia were about to break forth. In a note to J. C. Arthur, the unfortunate mistake of calling the pustules aecia brought a prompt response from him that the fungus was *Puccinia pittieriana* P. Henn., a short-cycle form, but that "even good mycologists had made the same mistake." This extends the range of the rust considerably farther north than was previously known. The occurrence of rust at El Desierto is well known to such men as Professor Herrera in Mexico City, but no published record is known to the writer and, since he did not mention it, probably none is known to Doctor Arthur. In some cases the rust was very abundant and was doing obvious damage to the plants.

### BLIGHT

Typical potato blight was found on some plants of *Solanum antipoviczii* growing in a recent "fill" along the roadside at 59 km. from Mexico City on the improved road to Cuernavaca. The diagnosis was confirmed by microscopic examination. No record is known to the writer of the occurrence of *Phytophthora infestans* on wild plants in Mexico. Professor Herrera felt sure that a record existed, but, when he turned to the place where he expected to find it, was unable to do so. The occurrence of this organism in Mexico makes it understandable how it can be that there are growing in the highlands several tuberous species either immune from or very highly resistant to blight. *Solanum verrucosum* was sent to Switzerland shortly after the outbreak of blight in Europe and was at first considered free from "the disease," but, in a year of severe blight, went down like the local varieties. The species possesses a certain amount of resistance

when compared to the varieties that are commonly grown in North America, but, of all the species in Mexico, it is one of the few that could not give relief from the disease.

Blight was not seen on cultivated plants during the 10 weeks in Mexico for the very good reason that no cultivated plants were seen. Some potatoes are produced throughout this area and were seen in the markets. The 2 common varieties encountered had the appearance of very small Cobbler and Triumph, with the red variety predominating. These commercial sorts, when tested at Ithaca, proved to be susceptible.

### SPOT

The most interesting disease encountered on wild potatoes is one that is here called spot. The lesions occur abundantly on the foliage, and clumps of *Solanum demissum* were found at El Desierto that were nearly defoliated on this account. The very young spot has the glassy appearance of a young lesion caused by *Phytophthora infestans*. As it enlarges the spot has the appearance of the blight caused by *Alternaria solani*, but the characteristic target lines are absent. A good many spots may occur on 1 leaf, the intervening tissue turns yellow, and the whole leaf dies, but it usually clings to the stem by a shred. In this condition the plant exhibits the usual symptoms of late blight. This condition has been noted previously on *S. demissum* when grown in the greenhouse at Ithaca, and it has been suggested elsewhere[3] that this is the disease Lindley and later Klotzsch called "the disease," namely, blight. Spot has appeared abundantly on *S. demissum* and some other Mexican species when grown recently at Ithaca. The most surprising thing about the disease is the fact that no organism can be found in the tissue. Microscopic examination reveals nothing, and platings remain sterile. The parent plant may show severe spotting and the shoots that come up from long stolons in the same pot may be entirely free. For the present, spot must be considered of physiological nature the cause of which is not known.

### VIRUS DISEASES

The relatively cool climate of the *tierra fria* should make it possible to detect such diseases as mosaic on the wild plants at any time. No positive cases of mosaic were encountered. When tubers from the collections were planted at Ithaca, 2 wild plants and 3 plants of cultivated varieties gave indications of mild mosaic. An attempt to transmit the disease to susceptible varieties failed but needs to be tried again.

Leaf roll was not seen in the field and nothing resembling leaf roll or

[3] Reddick, Donald. Blight-resistant potatoes. Phytopath. **18**: 483–502. 1928.

any of the other virus diseases appeared in the greenhouse cultures. Just how these diseases appear on wild species must await the results of reciprocal inoculation tests—wild plants to healthy cultivated varieties of known reaction; diseased cultivated to wild plants.

### TUBER DISEASES

No diseased tubers were found on wild plants, but some tubers in the market were seen that seemed to have the sclerotia of Rhizoctonia on them.

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## PHYTOPATHOLOGICAL NOTE

*Macrosporium solani on tomato fruit.*—Rosenbaum showed in 1920 that "nailhead spot" was due to a fungus that had smaller spores than *Macrosporium solani* and that produced no wine-red coloration in agar cultures, and he ascribed this fungus to the species *M. tomato.* Nevertheless, in several subsequent publications nailhead spot has been attributed to *M. solani.*

*Macrosporium solani* is becoming common in tomato greenhouses in South Australia but has never been found causing a nailhead spot. The fungus can be exceedingly severe on the leaves without causing any injury to the fruit. Figure 1, however, shows the symptoms that were observed in one case of fruit injury that came under notice. The grower stated that "individual tomatoes in a bunch seemed to get it, leaving the remainder of the bunch untouched. The diseased ones were very noticeable as they started to color long before the rest of the bunch."

FIG. 1. Lesions caused by *Macrosporium solani* on the stem and the calyx end of Early Dwarf Red tomato fruit.

Most of the infections started either at the stem end or at the calyx end while the fruit was still green, but in a few cases the spots were lateral. The lesions were rather hard, sunken, and slowly spreading on green fruit, but when specimens were kept in a moist dish for about a fortnight the mycelium would spread slowly through the ripening fruit, causing a soft rot with dark brown discoloration.

McWhorter,[1] who had considerable material under observation, figures the black stem-end lesions produced by the fungus on the fruit, stating that it leads to dropping of the fruit, but he makes no mention of nailhead

[1] McWhorter, F. P. The early-blight diseases of tomato. Virginia Truck Exp. Sta. Bul. 59. 1927.

spot as a symptom. It would appear probable, therefore, that the old idea that *Macrosporium solani* can cause nailhead spots on tomato fruit is definitely erroneous.—GEOFFREY SAMUEL, Waite Agricultural Research Institute, University of Adelaide, South Australia.

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